PROCEEDINGS OF THE 11TH YSF SYMPOSIUM

JANUARY 27, 2023

Young Scientists Forum

National Science and Technology Commission

11TH YSF SYMPOSIUM

27th January 2023

Organized by

Young Scientists Forum

National Science and Technology Commission

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FULL PAPERS

Message from the Acting Director, National Science and Technology Commission

With great pleasure, I convey this message as the Acting Director / CEO of the National Science and Technology Commission (NASTEC) at the inauguration of the 11th YSF Annual Research Symposium organized by the Young Scientists Forum (YSF) of NASTEC.

YSF Symposium, initiated in 2012, has been held successfully since its inception, and the 11th symposium will be held in 2023. This year the symposium is focused on promoting Research & Development with relevant Interventions identified under the ten thrust areas of the National Research & Development Framework (NRDF), which was developed by the NASTEC and in consultation with a large number of relevant experts in the scientific community. Young Scientists & promising researchers of the country are encouraged to conduct research under NRDF's ten thrust areas, where the first 5 areas cater to improving quality of life. At the same time, the latter 5 contribute to the country's economic development. I am pleased to note the number of research publications related to thrust areas of Health, Food, Nutrition & Agriculture, Environment, Energy, Basic Sciences, Emerging Technologies & Indigenous Knowledge, and ICT being presented in this symposium.

It is my fervent hope that the young scientists gathered at this symposium will not only gain knowledge of the novel research ideas presented but also will mobilize their collective intellect to provide S&T solutions in order to overcome the challenges faced by the country.

I wish you good luck with the proceeding of the sessions and future endeavors of YSF in relation to S&T development.

Mr. Janaka S. Geekiyanage

Act. Director/CEO
National Science and Technology Commission

Message from the Steering Committee Chairperson

Young Scientist Forum

Sri Lanka is a country with thousands of beautiful natural sights and millions of beautiful minds. At the time we are publishing the proceedings of the 11th YSF Symposium, the country is in a need of young beautiful minds who conduct research and do innovations to make Sri Lanka a country with a production-based economy. The country needs the involvement of young scientists to raise again as a strong nation. At such instance, Young Scientists Forum (YSF) of the National Science and Technology Commission (NASTEC) has been a place for young academics, researchers, and scientists to advance their scientific careers and serve the country to save the country. The YSF Symposium was initiated with the objectives of inculcating quality research practices, identifying young researchers while giving due recognition and encouragement, and expanding the exploration of unseen areas of science and technology.

The undoubtedly challenging era we are passing as a nation has urged the necessity of emerging young scientists who identify novel strategies to assure a safe future for coming generations. Accordingly, 11th YSF Symposium is organized with the theme of "Integration of Science, Technology, and Innovation for a Better Sustainable Economy". Under this theme, the researchers would find ample opportunities for exploring new knowledge within the areas of Basic Sciences, Emerging Technologies, Indigenous Knowledge, Information Communication Technology, Food, Nutrition, Agriculture, Environment, Energy, and Health. We believe that the symposium will open new avenues in developing an insightful forum and aspiring for excellence in research utilizing diverse aspects of science and technology in the way forward of crisis.

It is my honor and privilege to be involved in 11th YSF Symposium, contributing towards its continued success since 2017. At this proud moment, I would like to convey my sincere gratitude to Prof. Veranja Karunaratne, the Chairman of NASTEC and to Mr. Janaka S. Geekiyanage, the Acting Director of NASTEC for their invaluable guidance and continued support for all the YSF activities including the 11th YSF Symposium. I am indeed honored to have the contribution of the panel of reviewers and evaluators to make the success story of the Annual Symposium. The appreciative efforts of the dedicated and hardworking Steering Committee of the YSF are behind the accomplishment, which you are today experiencing. I wish to thank the Editorial Board for their untiring efforts in producing the Proceedings of the Symposium. A special appreciation goes out to Ms. Thilini Munagamage, Scientist of NASTEC and the Symposium Coordinator,

for her efforts in all aspects of YSF activities. I wish all the success to the YSF for its pathway to strengthen, build and guide the Young Scientists in Sri Lanka.

Mr. Akila Jayasanka
The Chairman, Young Scientists Forum

Foreword from the Editors

It is with great pleasure, the Young Scientist Forum (YSF) present the proceedings of the 11th YSF Symposium. The annual research symposium of the YSF provides an ideal opportunity for the local young scientists to share the research interests in various disciplines and to initiate cross-disciplinary collaborations. It is a place of networking, where constructive scientific feedback is mostly nurtured.

Out of the 60 submissions received as extended abstracts and full papers for the year 2023, 50 submissions were selected through a double-blind review process. The materials submitted by the authors were reviewed by two expert reviewers in the relevant field and have been edited by editorial board of the YSF. The views expressed in extended abstracts and full papers remain the responsibility of the named authors.

We would like to express our gratitude to all contributing authors for sharing their outstanding research findings and for the panel of reviewers for invaluable feedback to enhance the quality of this publication. The editorial board is very much thankful to Mr. Janaka Geekiyanage, the Acting Director of the NASTEC for funding and facilitating the events of YSF with great enthusiasm. NASTEC coordinator Ms. Thilini Munagamage and NASTEC staff, and the members of the YSF Steering Committee are also acknowledged for the immense support rendered in organizing the symposium and compilation of the proceedings.

We wish the 11th YSF symposium a great success and extend warm wishes to all the authors.

The Editorial Board

11th YSF Symposium Proceedings

EXTENDED ABSTRACTS

FOCUS AREA

Basic Sciences, Emerging Technologies & Indigenous Knowledge

In silico studies of antiviral property of Paspanguwa

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Introduction

SARS-CoV-2 was found as a new type of coronavirus by the WHO in early 2020. The disease soon spread throughout the globe. They are called "coronavirus" because they have crown-shaped spike proteins on their outer membrane surfaces. COVID-19 is a disease produced by the SARS-CoV-2 virus which can cause a respiratory tract infection. Out of seven coronaviruses, SARS-CoV-2 can cause serious illnesses. Several variations of SARS-CoV-2 are now circulating, some of which are proving to be deadly. They are Alpha, Beta, Gamma, Delta, and Omicron.[1] The SARS-CoV-2 virus connects to the ACE2 receptor utilizing a spike-like protein present on its surface, similar to a key put into a lock, it stops ACE2 from doing its role of regulating angiotensin II signaling, this cause more angiotensin II to injured tissues.[2] In this research, Phytochemicals contained in the' Paspanguwa' water extract were found based on the literature review carried out. The above ligands were docked to the SARS-CoV-2 spike protein binding site to the ACE2 receptor protein by considering all variants of the virus and their interactions with the ACE2 receptor, to predict whether there is a probability of chosen ligands to interfere the interaction between spike protein and ACE2 receptor using computational chemistry by finding the binding energy and binding residues.

Materials and Methods

Materials

Hardware- Intel Core i5 7200U (2.50-3.10GHz, 4GB DDR4, 1TB) laptop.
Software's and Web servers - Avogadro, SWISS-MODEL, AutoDock Tools 1.5.6, AutoDock 4.2, BLAST Server, UniProt align server, SAVES v6.0 server, ProSA-webserver, Protein-ligand-interaction-profiler, LigPlot, Pymol, Openbabel, SwissADME-web server, Galaxy Refine-GalaxyWEB server.

Methods

Ligands identification

Based on the literature reviews sixty ligands were found. Then the Canonical SMILES of each ligand were obtained from the PubChem database. Above Canonical SMILES were subjected to the SwissADME web server to check their

drug-likeness. Ligands that followed Lipinski's rule of five were selected using this web server.

Ligands preparation

Based on the ligands identified, 3D structures of ligands were downloaded from the PubChem database in SDF format. Then the above files were subjected to Avogadro software and geometries were optimized using Force Field MMFF94, algorithm steepest descent, 500 steps. Then the above files were saved in PDB format. Then the PDB format files were subjected to AutoDock Tools 1.5.6 and converted to PDBQT file format.

Protein preparation

First based on the literature reviews, UniProt ID Q9BYF1 was found for the human ACE2 receptor. Then the protein sequence of the ACE2 receptor was obtained from the UniProt website for the UniProt ID Q9BYF1. That protein sequence was run via the BLAST server to find the homology sequences and thereby highest percentage sequence similarity PDB ID was found. The above protein sequence was also subjected to SWISS-Model online modeling server to find the template with higher GMQE, sequence similarity, and higher sequence coverage. The above template should also parallel with the BLAST parameter. UniProt align server was used to align the protein sequence with modeled sequence and determine whether active sites and spike protein binding sites were conserved. Then the template follows the above facts used to build the protein model.

Model validation and Refinement of model structure

After modeled protein was built, the Refinement was done by Galaxy Refine in the GalaxyWeb server. Then the protein is subjected to ERRAT, verify 3D and PROCHECK contain in the SAVES v6.0 server and also the ProSA server to validate the modeled structure.

Molecular Docking

Water molecules were deleted, Polar hydrogen atoms added and finally, Kollman chargers were added to the modeled protein structure using AutoDock Tools 1.5.6. Then the above file is saved as a PDBQT format file. By detecting torsion root using AutoDock Tools 1.5.6, ligand files were transformed to PDBQT format files. Ligands were docked into the 3D structure of a protein by drawing the grid on the binding site. Due to the grid box contained in the binding region, Autogrid 4.2 (.gpf) files were generated. Grid parameter files and map files generated by Autogrid 4.2. Lamarckian genetic algorithm (LGA) was utilized for docking studies and the parameters were adjusted as follows; number of genetic algorithms (GA) runs:20, the maximum number of energy evaluations; 5000000, population

size;150, a maximum number of 27,000 generations and other settings were not changed. AutoDock 4.2 (.dpf) utilized for docking. The ligands were set to flexible and the protein adjusts to rigid. Finally, the resultant files were generated as (.dlg).

Analysis of docking results

AutoDock Tool 1.5.6. was used to analyze binding energies. Interactions between protein-ligand complexes and amino acids located in the binding pocket were analyzed using a protein-ligand interaction profiler. LigPlot is used to get 2D images related to interactions and 3D images with interactions obtained using pymol.

Results and Discussion

Ligands identification -The main phytochemicals from each five ingredients of herbs contained in 'Paspanguwa', that were extracted to water using various extraction techniques were found based on literature reviews. Those phytochemicals were again filtered into 36 ligands based on their percentage amount extracted to water from each five ingredients and also with their drug-likeness properties.

Protein preparation - The protein sequence of the ACE2 receptor was submitted to SWISS-Model, an online modeling server, to discover the template with the highest GMQE, sequence similarity, and sequence coverage. The template obtained from the SWISS-Model with 0.81 GMQE was matched with the highest percentage sequence similarity PDB ID obtained from protein blast. After modeled protein was built, the Refinement was done by Galaxy Refine in the GalaxyWeb server. Then it provides five models, model with the highest RMSD (0.392) was selected.

Model validation – VERIFY 3-D server - The model's quality was determined by the score of the passed residues, which was larger than 0.2. According to the results, modeled protein has 95.64% residues averaged 3D-1D score greater than 0.2. ERRAT server - ERRAT server was used to evaluate the statistics of interactions, which are non-bonded between various atom types. Normally scores of more than 60 are considered acceptable. The modeled protein score was 94.6401. PROCHECK. Server-The Ramachandran plot was used to examine the placement of amino acid residues in the permitted and forbidden zones, and also the protein structure's overall stereochemical quality. According to theresults, modeled protein is 96.3% in the favored region, signifying that the structure is reliable. ProSA Server- ProSA is a standard tool for detecting errors in 3-D protein structure models. The z-score reveals how successful a model is

overall. The modeled protein's ProSA score was -13.61, confirming its validity. Protein-ligand interaction analysis - The binding energies for each protein-ligand complex were retrieved using AutoDock 4.2 and they are shown in the below table.

Table 1. Docking results

Compound	Ligand	Binding energy (kcal/mol)
Pathpadagam	Urosilic Acid	-9.23
	6- α -hydroxygeniposide	-5.59
	Asperulosidic acid	-5.36
	Biflorin	-6.35
	γ-sitosterol	-10.11
	Geniposide	-6.63
	Oleanolic acid	-8.42
Coriander	Linalool	-5.69
	Limonene	-6.08
	Geranyl Acetate	-5.37
	Geraniol	-5.58
	γ -terpinene	-5.67
	Cineole	-6.52
	Camphor	-5.64
	α -pinene	-6.39
Ginger	6-Gingerol	-5.04
	zingiberene	-5.98
	β -bisbolene	-5.97
	α -curcumenne	-6.22
	6-shogaol	-5.95
	6-paradol	-5.87
	Diosgenin	-9.18

Venivalgata	Berberine	-7.60
	Berberrubine Chloride	-6.62
	Jatrorrhizine	-6.54
	Palmatine	-6.61
	Sitosterol glucoside	-8.35
	Thalifendine	-6.57
	Berberrubine Chloride	-6.62
Katuwalbatu	Stigmasterol	-10.19
	Apigenin	-6.75
	Carpesterol	-10.42
	Coumarin	-5.97
	Diosgenin	-9.18
	Linoleic Acid	-4.65
	Oleic Acid	-4.10
	Solasodine	-10.34

The above docking results were obtained for site-specific docking by considering all types of variants in SARS-CoV-2 spike protein interactions with ACE2 receptor protein and the grid box was drawn to that site. Thirty-six ligands were docked to the site mentioned in Table 1 and out of that 20 ligands' binding energy was greater than (BE) (-6.00 kcal/mol). Stigmasterol, Solasodine, Carpesterol, and γ-sitosterol ligands binding energy to ACE2 receptor protein was greater than (-10.00 kcal/mol). According to the results, higher negative binding energies reveal stable protein-ligand complexes. According to the interactions between ligands giving binding energy greater than -7.00 kcal/mol with spike protein of SARS CoV2 binding site in ACE2 receptor, only ARG393 is involved. The above results indicate that ligands mainly bind to other site amino acids included in the grid box. An area where a grid box is drawn covers the allosteric site2 and allosteric site3 in the ACE2 receptor. Drugs targeting allosteric sites, bind to places other than the enzyme's active site and frequently allosterically change the protein's conformation. Certain evidence suggests that ACE-2 active site blockers fail to stop the SARS-CoV-2 infection from progressing because amino acids responsible (H345, H505, and R27313) for binding to blockers are located in the core of ACE-2 protein.ACE-2's allosteric site 1 is immediately beneath its active site. The

allosteric sites 2 and 3 are situated adjacent to the interacting amino acid residues, which are normally involved in hydrogen bonding with the SARS-CoV-2 receptor-binding domain. According to the interactions between ligands giving binding energy greater than -7.00 kcal/mol, almost all the ligands binding to certain amino acids contain in allosteric site2 and allosteric site3.

Conclusions and Recommendations

According to the interactions between ligands giving binding energy greater than -7.00 kcal/mol, almost all the ligands binding to certain amino acids contain in allosteric site2 and allosteric site3. The other 13 ligands' binding energies were greater than (BE) (-6.00 kcal/mol) and may have tended to bind to allosteric sites. So, the contribution of each ligand may impact interactions between spike protein domains and the ACE2 receptor by binding to the allosteric sites 2 and 3. Therefore, the above results indicate there is a probability to cure COVID-19 by consuming Paspanguwa.

Results obtained for ligands giving binding energy greater than -7.00 kcal/mol, should be identified at the compound level by LC-MS/MS, LC-MS, and also further confirm using phytochemical screening. Molecular dynamics studies of the complexes will be carried out in future to support the results of the present study.

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FACILE BIOGENIC SYNTHESIS OF SILVER-ZINC OXIDE NANOCOMPOSITES USING *Borassus flabellifer* PULP AND SPROUT EXTRACTS AND EVALUATION OF THEIR ANTIOXIDANT ACTIVITIES

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Introduction

Palmyra (*Borassus flabellifer* L.) is an Arecaceae plant that grows in drier regions of the world. The palmyra tree grows widely in northern Sri Lanka, and the fruits mature in August and fall off from the tree between September and October Palmyra is a medicinally important plant, containing biological and pharmacological value [1]. Palmyra fruit is high in essential amino acids as well as bioactive compounds which have antioxidant properties. Palmyra sprouts are rich in calories, a good source of vitamin E [2], and have antioxidant properties. Oxidative metabolism is essential for life's survival, and such antioxidants protect the body against reactive oxygen species (ROS) [3].

Plant-mediated nanocomposites (NCs) development is a revolutionary technique with numerous advantages over physicochemical methods. Plants contain a large number of biologically active primary and secondary metabolites due to their high genetic variability. They also aid in the bioreduction of ions to the nanoscale, as well as the capping of nanoparticles (NPs), which is critical for stability and biocompatibility [4]. The green approach has the added benefit of increasing the life span of nanomaterials (NMs) due to the immense properties of plant-mediated NPs. Hence, in recent years, there has been considerable attention and advancement in nanomaterials synthesis via the green approach. Ag NPs and ZnO NPs synthesis is the most commercialized research area due to their versatility and has been used in several industrial applications.

Hence, in this study, we report a simple and environmentally friendly method of synthesizing Ag-ZnO NCs using aqueous palmyra pulp and sprout extracts as the capping and stabilizing agents and compared the antioxidant potential with reference standards and the plant extracts. Although plant-mediated Ag-ZnO NCs synthesis has previously been reported, there is a scarcity of literature on palmyra pulp and sprout-mediated Ag-ZnO NCs and their antioxidant activities [5].

Materials and Methods

Preparation of plant extracts

The mature palmyra fruits were collected from the trees near Kilinochchi area, and the palmyra sprouts were collected from the local market at Dehiwala. The mature fruit pulp was obtained after squeezing the pulp using muslin cloth without fiber. The obtained pulp was freeze-dried. The dried pulp sample (10 g) was dissolved in 200 mL of distilled water, and the mixture was continuously stirred under 70 °C for 30 minutes to obtain the pulp extract. The sprouts were washed thoroughly and then washed with distilled water. The sprouts were cut into thin slices and oven dried at 45 °C for three days. The dried sprout sample was powdered using an electrical grinder. The powdered sample (10 g) was added to 200 mL of distilled water and continuously stirred under 70 °C for 30 minutes to obtain the sprout extract. The aqueous extracts were filtered through Whatman No.1 filter paper. The collected extracts were then refrigerated for future experiments.

Optimization the conditions for Ag-ZnO NCs synthesis

The green synthesis of Ag NPs and ZnO NPs was carried out by mixing aqueous pulp and sprout extracts with different concentrations of $AgNO_3$ and $[Zn(CH_3COO)_2]$. $2H_2O$ solutions (0.05M, 0.01 M, 0.1 M) in different ratios (aqueous extract: ion precursor) for both NPs such as 1:1, 1:3, 2:5 and 1:9 only for ZnO NPs synthesis. The effect of irradiation methods was assessed by varying the different irradiation sources such as solar, microwave, and UV. The pH of medium was changed for the synthesis of ZnO NPs by varying the pH 7, 10, and 12. To determine the effect of time period on NPs synthesis, reactions were performed at different time intervals (0.5–24h). Based on the optimized conditions sufficient yield of NMs was obtained. The resultant mixtures were centrifuged (4500 rpm) for 15 minutes. The residue was washed with deionized water until the supernatant appeared colorless. The residue was oven dried at 70^{o} C for eight hours to obtain Ag NPs.

Based on the optimized conditions preparation of Ag-ZnO NCs carried out through following synthesis procedure. Initially ZnO NPs were prepared by adding 10 mL of plant extract drop wise into 90 mL of $[Zn(CH_3COO)_2].2H_2O$ solutions (0.01 M) with continuous stirring. Meanwhile 2M NaOH was added until the pH of the solution reached 12. The resultant mixture was stirred continuously (2 hrs) and incubated (24 hrs) under dark to obtain the dried ZnO NPs. Subsequently the plant extract (5 mL) was added to drop wise into 15 mL of $AgNO_3$ (0.05 M) solution and subjected to solar irradiation for 30 minutes. Previously prepared finely powdered ZnO NPs (50 mg) were added to above solution under continuous stirring for 30 min. The resultant mixture was kept in dark for 24 h. The incubated mixture of NCs was centrifuged at 4500 rpm for 15 minutes and the collected precipitate was washed with distilled H_2O three times. The residue was oven-

dried at 70 ^{o}C for 12 h, and the obtained NCs were preserved in airtight vials for further studies.

Characterization of phytogenic NMs

Surface plasma resonance (SPR) peaks of NMs were determined by wavelength range of 300-700 nm. The functional groups of phytochemicals present in the plant extracts used as reducing and capping agents for the synthesis of NMs were determined using an FTIR spectrometer in the 500 – 4000 cm^{-1}. Morphological analysis of NMs was carried out using SEM analysis. The sizes of synthesized NMs were determined through TEM analysis. For the structural analysis of the synthesized NMs, an X-ray powder diffractometer was used.

Antioxidant potential of phytogenic NMs

Standard procedures for DPPH, ABTS, and FRAP assays were used to determine free radical scavenging activity and potential antioxidant power of biogenic NMs. The antioxidant activity of green synthesized NPs was compared with that of plant material.

Results and Discussion

The UV-Vis absorption peak was observed at the range 350-360 nm confirming the intrinsic bandgap of ZnO NPs and 437-440 nm for the formation of Ag NPs. There were two peaks between 350 nm and 450 nm that indicted the SPR peaks for Ag-ZnO NCs.

FTIR spectrometry was performed to identify the functional groups associated with the palmyra pulp and sprout mediated synthesized Ag, ZnO NPs and Ag-ZnO NCs. The FTIR spectra of the plant extracts revealed the presence of significant functional groups that responsible for metal ion reduction and acting as reducing and capping agents for NMs synthesis. The synthesis of hexagonal phase of ZnO and formation of tetrahedral coordinated Zn was indicated by the peaks at frequency range 710-900 cm^{-1}.

The shape, size, and aggregation state of biogenic NMs were depicted using SEM analysis. Figures 1 and 2 show the formation of spherical shaped Ag NPs, nanoflower shaped ZnO NPs, and both spherical and nanoflower containing Ag-ZnO NCs synthesised palmyra pulp (PPulp), and palmyra sprout (PSprt) materials under optimal conditions and confirmed that the average particle sizes are within the nano range.

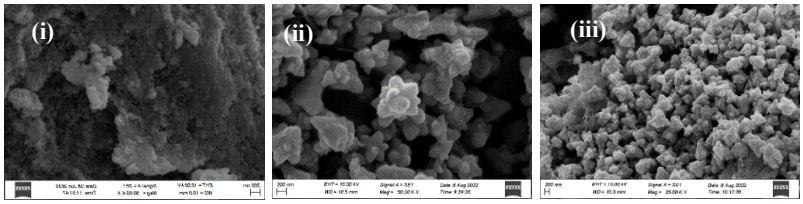

Figure 1. SEM images of (i) AgNPs, (ii) ZnO NPs, (iii) Ag-ZnO NCs synthesized from PPulp and showing their morphologies

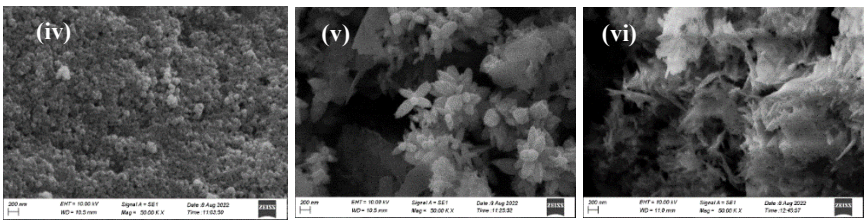

Figure 2. SEM images of (iv) AgNPs, (v) ZnO NPs, (vi) Ag-ZnO NCs synthesized from PSprt and showing their morphologies

TEM image of Ppulp mediated Ag NPs, ZnO NPs and Ag-ZnO NCs with particle sizes were 17.06 nm ± 2.31 nm, 91.85 ± 13.39 nm, and 17.88 ± 3.62 nm respectively and PSprt mediated Ag NPs, ZnO NPs and Ag-ZnO NCs with particle sizes was 12.66 ± 3.26 nm, 97.87± 18.92 nm, and 15.32± 4.44 nm respectively.

The XRD patterns of ZnO NPs prepared from Ppulp and Psprt extracts indicated the typical hexagonal wurtzite structure of ZnO NPs and showed characteristic peaks positioned at 2θ values of 31.84°, 34.52°, 36.38°, 47.64°, 56.70°, 63.06°, 67.10°, 68.10°, and 69.18° which are indexed as (100), (002), (101), (102), (110), (103), (200), (112) and (201) planes, respectively. The XRD spectra with the main characteristic peaks at 2θ values of 38.19°, 46.18°, 67.44°, and 77.70° matched with (111), (200), (220), and (311) planes of Ag, respectively. All the peaks in the XRD pattern can be readily indexed to a face-centered cubic structure of Ag that confirms the presence of Ag. Other prominent peaks in the XRD spectra indicated that organic compounds come from bioactive compounds in plant materials, aside from these characteristic peaks for both ZnO and Ag formation. XRD patterns for Ag-ZnO NCs indicated that both characteristic peaks were obtained from ZnO and Ag samples.

The results revealed that pulp and sprout mediated NMsscavenged DPPH and $ABTS^{\bullet+}$ free radicals in a concentration dependent manner.

Table 1. IC_{50} values of pulp and sprout mediated NMs

	Sprout mediated NMs				Pulp mediated NMs				Standards	
	Ag NPs	ZnO NPs	Ag-ZnO NCs	Sprout	Ag NPs	ZnO NPs	Ag-ZnO NCs	Pulp	Ascorbic acid	Trolox
DPPH IC_{50} value (ppm)	62.63	96.98	64.36	127.58	73.68	77.94	74.36	144.79	59.23	
$ABTS^{\bullet+}$ IC_{50} value (ppm)	92.25	112.56	56.01	125.48	120.21	170.13	96.51	192.66		50.65

Table 1 shows the IC50 values of the DPPH and ABTS•+ radical scavenging assays for pulp and sprout mediated NMs. Palmyra sprout mediated NMs had a higher potential to scavenge ABTS•+ and DPPH free radicals, and the FRAP assay also revealed that NMs have a higher antioxidant potential than their respective plant materials. Furthermore, it is clear that the antioxidant activity is size dependent, as the sprout mediated synthesised NPs and NCs have smaller particle sizes than the pulp mediated. When the individual NPs are considered, the NCs have a significantly higher antioxidant potential.

Conclusion and Recommendation

Palmyra pulp and sprout were used to successfully biosynthesize Ag-ZnO NCs. FTIR analysis revealed that phytochemicals found in palmyra pulp and sprout exracts, such as phenols, flavonoids, and primary metabolites, acted as reducing and capping agents. The average particle size of palmyra sprout-mediated synthesised Ag NPs was smaller, according to SEM and TEM analysis. The XRD analysis confirmed the formation of pure Ag NPs and ZnO NPs, with distinct peaks indicating a face-centered cubic structure for Ag and a hexagonal structure for ZnO NPs. The pulp and sprout-mediated Ag-ZnO NCs displayed high DPPH, $ABTS^{\bullet+}$ radical scavenging capacities and FRAP inhibition power than the respective plant extract and individual NPs, indicating that the sprout extracts contain excellent reducing and capping agents for the biogenic synthesis of smaller size NPs and NCs. The current findings revealed that the green synthesized Ag-ZnO NCs have the potential antioxidant capacity, and the ability increased with decreased NP size.

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MODELING AND FORECASTING CINNAMON EXPORT IN SRI LANKA

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Introduction

Cinnamon is one of the oldest and popular spices in Sri Lanka which played a major role in ancient Ceylon history. Sri Lanka has maintained a noticeable income over the Cinnamon industry at the global level. The main difficulties in the Cinnamon industry of Sri Lanka are lack of skilled labour, high cost of labour & planting materials, and unstable prices in the market. Planters, cinnamon factories, investors, exporters, policy makers and government are interested in knowing the future income of Cinnamon. Considering these factors necessity of a forecast relating to the Cinnamon exports in Sri Lanka is encouraged. This study's main objective is to identify the behavior of the income obtain through the Cinnamon exports and forecast the future values. Therefore, it is mainly inspired to investigate the behavior of Cinnamon export income over the past ten years in Sri Lanka and conduct a forecast for future export incomes. By identifying trends in Cinnamon export, the government can make farmers aware of the requirements in the export industry by providing infrastructure facilities. Sri Lankan Cinnamon exporters and potential investors can determine whether long-term investments will be beneficial or not in the Cinnamon export industry in Sri Lanka.

Research has been conducted to forecast Cinnamon export prices in Indonesia and Vector Autoregressive (VAR) model has been employed for forecasting purposes [1]. Sarawak Black Pepper Price in Malaysia has been forecasted by applying the ARIMA model [2]. Even though there are many pieces of research related to predicting the price of spices, there is a research gap for assessing their export income. Moreover, attention has not been paid to forecasting the export income of cinnamon in Sri Lanka. This study would be beneficial to fulfill the aforementioned research gap.

Materials and Methods

The univariate time series analysis approach was performed for the monthly income of Cinnamon exports in Sri Lanka. Data from July 2011 to June 2021 were extracted from the monthly bulletins published by the Central Bank of SriLanka [3].

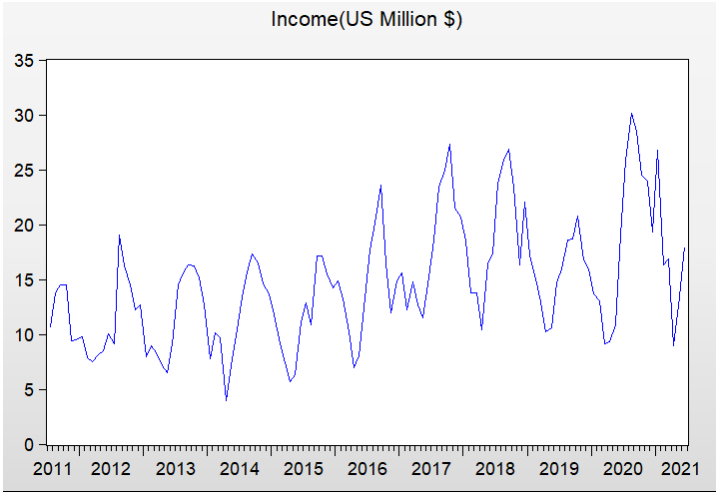

Figure 1. Time Series plot of monthly income of Cinnamon exports

The first 95 observations (Nearly 80%) from 120 data points were allocated for the model fitting purpose and post-sample (latest 20%) for assessing the performance of the identified model. Trend and seasonal variation in the line chart encouraged to model employing the Seasonal Autoregressive Integrated Moving Average (SARIMA) process [4].

The stationarity of the data was checked using ADF (Augmented Dickey-Fuller), PP (Phillips-Perron), and KPSS (Kwiatkowski–Phillips–Schmidt–Shin) tests. Differencing and log transformation were applied to eliminate the trend and high variance respectively. The seasonal factor was detected using the correlogram of the transformed series. After performing seasonal difference transformation, the Autocorrelation Function (ACF) plot and Partial Autocorrelation Function (PACF) plots were created for the stationary time series and cut-off lags in ACF and PACF plots were detected. Candidate models were suggested with the detected cut-off lags/ significant lags. Once fitting the candidate models, the better model was detected using the minimum in Akaike's Information Criterion (AIC). Assumptions of heteroscedasticity, autocorrelation, and normality were assessed using the ARCH test, Ljung-Box Q test, and Jarque-Bera test respectively. After verifying the satisfaction of assumptions, the reserved dataset was used for checking the performance of the identified model. Root Mean Squared Error (RMSE), Mean Absolute Error (MAE), and Mean Absolute Percentage Error (MAPE) were used to measure the performance of the fitted model. Table 1 was used to conclude the level of accuracy of the model [5].

Table 1. MAPE and level of accuracy

MAPE	Level of Accuracy
< 10%	Highly Accurate
11% to 20%	Good Forecast
21% to 50%	Reasonable Forecast
> 51%	Inaccurate forecast

Results and Discussion

When evaluating the stationarity of the log transformed and first differenced time series, ADF and KPSS tests indicated non stationarity while PP test denoted stationarity at 5% level of significance. The Autocorrelation Function (ACF) plot of the transformed (differenced and log transformed) series indicated a seasonal pattern of 12. Aforementioned unit root tests were performed again on the seasonal differenced series and all three tests indicated the stationarity of the series at a 5% level of significance.

Table 2. Results of tests for stationarity

	Test	Value
ADF	p-value	0.0000
PP	p-value	0.0001
KPSS	Test statistic	0.2969

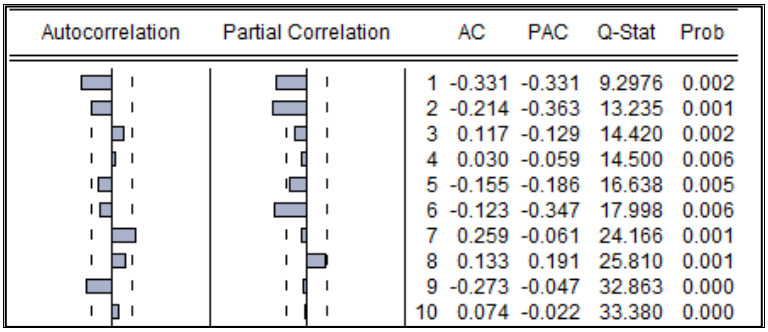

Figure 2. ACF and PACF plots

According to the significant lags of ACF and Partial Autocorrelation Function (PACF) plots, six seasonal ARIMA models were identified as candidate models.The suggested candidate models were reduced to two models due to the significancy of the coefficients of parameter terms. Table 2 illustrates all considered significant models along with AIC values.

Table 3. AIC values of the significant models

Models	AIC value
ARIMA (0,1,1) (0,1,0) $_{[12]}$	-0.044
ARIMA (1,1,2) (0,1,0) $_{[12]}$	-0.055

ARIMA (1,1,2) (0,1,0) $_{[12]}$ model which had the minimum AIC value, was selected as the better model. The selected model satisfied all three assumptions based on the results obtained from statistical tests as illustrates in Table 3. The Table 4 summarizes the performance measures of the adequate model.

Table 4. Results of the adequacy checking of the fitted model

Assumption	Test	p-value	Decision
Heteroscedasticity	ARCH test	0.9448	satisfied
Autocorrelation	Ljung-Box Q test	Most of the p-values are greater than 0.05	satisfied
Normality	Jarque-Bera	0.6477	satisfied

Table 5. Performance measures of the fitted model

Performance Measure	Value
RMSE	5.007
MAE	3.982
MAPE	21.065

According to the Table 1, accuracy of 21.06% (nearly 20%) of MAPE value has an approximately good forecast. Further, the RMSE and MAE values of the selected model were considerably low values. Therefore, ARIMA (1,1,2) (0,1,0) $_{[12]}$ can be used to forecast the monthly income of Cinnamon exports in Sri Lanka. Figure 1 shows the extracted actual value vs forecasted value graph of the ARIMA (1,1,2) (0,1,0) $_{[12]}$ model for the test set. It can be observed that the fitted model has fairly captured the pattern of the actual data.

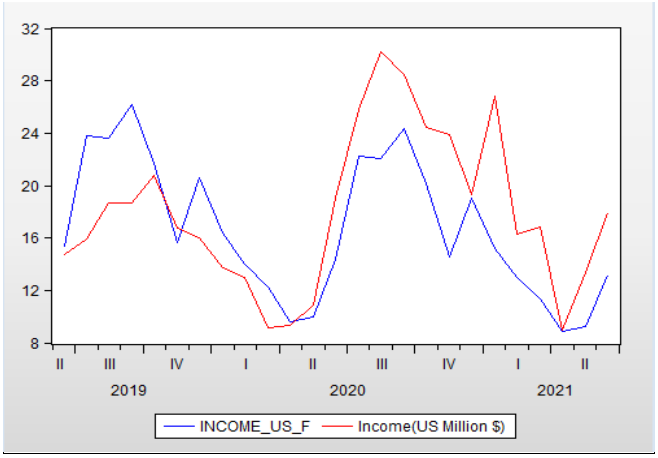

Figure 3. Actual values (Income (US Million $)) vs fitted values (INCOME_US_F) graph of fitted ARIMA (1,1,2) (0,1,0) [12] model

Conclusions and Recommendations

The main purpose of this analysis was to stabilize a univariate time series model to forecast the income of Cinnamon exports in Sri Lanka as Cinnamon has maintained a noticeable income. ARIMA (1,1,2) (0,1,0) $_{[12]}$ model was suggested to forecast the monthly income of Cinnamon exports in Sri Lanka with considerably low RMSE, MAE, and MAPE values. This model would benefit policymakers, planters, cinnamon factories, and exporters in identifying current trends in the income of Cinnamon exports. Since there were deviations of forecasted values from the actual values, it is better to find other factors affecting the income of Cinnamon exports in Sri Lanka along with multivariate analysis for modeling and forecasting. Moreover, according to the past literature, this analysis can be expanded using data mining techniques to suggest a more accurate forecasting model.

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In Silico Studies of Antiviral Property of Sri Lankan Curry Powder Against Norovirus G11-4 Genotype.

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Introduction

Norovirus (NoV) is a contagious, single stranded RNA virus belonging to the family *Caliciviridae.* Noroviruses are now classified into ten genogroups (GI-GX) and 48 genotypes. Among these groups, GII has been found to be the most pandemic strain that affects human health and GII.4 is the most common genotype that has been intensively studied in clinics. *NoVs* are a group of non-enveloped viruses but encapsulated by an icosahedral protein capsid that primarily cause acute gastroenteritis, of which most common symptoms are diarrhea, vomiting, nausea, stomach pain.

The capsid protein is divided into two major domains, the shell (S) and the protruding (P) domains, each forming the interior shell and the arch-like protrusions of NoV capsid, respectively. The P domain can be further divided into VP1 and VP2 subdomains. Human NoVs are also highly diverse and have multiple receptor binding patterns. NoVs recognize human histo-blood group antigens (HBGAs) types A, B and H, secretor and Lewis antigens as receptors or attachment factors. The P domain plays an important role in host immune response and receptor recognition because when VP1 binds with receptor molecule it is important to penetrate into the host cell during infection. Therefore, interaction between VP1 and receptors can be one of the most effective strategies in developing therapeutics against virus infection.[1]

In this research, Phytochemicals contained in the 'curry powder' water extract were found based on the literature carried out. The above ligands were docked to active site in P domain from norovirus strain saga4 in complex with HBGA. In order to predict whether there is a probability of chosen ligands to interfere the interaction between P domain from norovirus strain saga4 in complex with HBGA using computational chemistry by finding the binding energy and binding residues.

Materials and Methods

Materials

Hardware-Intel Core i5 7200U (2.50-3.10GHz, 4GB DDR4, 1TB) laptop. Software's and Web servers -SWISS-MODEL, Avogadro[2], AutoDock Tools 1.5.6 [3], AutoDock

4.2[4], BLAST Server[5], UniProt align server[6], SAVES v6.0 server[7], ProSA-webserver[8], Protein-ligand-interaction profiler[9], LigPlot[11], Pymol[11], Openbabel[12], SwissADME-web server[13], Galaxy Refine-GalaxyWEB server[14].

Methods

Ligands Identification

Based on the literature reviews sixty ligands were found. Then the Canonical SMILES of each ligand were obtained from the PubChem database. Above Canonical SMILES were subjected to the SwissADME web server to check their drug-likeness. Ligands that followed Lipinski's rule of five were selected using this web server.

Ligands Preparation

Based on the ligands identified, 3D structures of ligands were downloaded from the PubChem database in SDF format. Then the above files subjected to Avogadro software and geometry optimized using Force Field MMFF94, algorithm steepest descent, 500 steps. Then the above files are saved as PDB format. Then the PDB format files are subjected to AutoDock Tools 1.5.6 and converted to PDBQT file format.

Protein Preparation

Initially obtained 3-dimensional protein structure (PDB ID: 10.2210/pdb4X0/pdb) was checked to see if there are any missing amino acids. If there were any missing amino acids, to add them fully automated protein structure homology modelling server, SWISS-MODEL was used.

Model validation and Refinement of model structure

A thorough literature survey was done to find the exact amino acids in the active site of the protein and protein structure was investigated to check whether each active site amino acid is in the correct position. The obtained amino acids in active sites were further checked using the GASS-WEB server. The refined PDB file was uploaded to the server and the most probable active site was found.

Molecular Docking

Protein and ligand files were loaded as pdbqt files to AutoDock Tool 1.5.6. Autodock 4.2 (.dpf) and Autogrid 4.2 (.gpf) file were generated. Autogrid 4.2 was used to generate grid parameter files and map files. The genetic algorithm parameters were set as follows, the number of genetic algorithms (GA) runs: 100, population size: 300, the maximum number of evaluations: 25000000 and the other settings were set to default values. Autodock 4.2 was used for docking and generating result file (.dlg) files were generated.

Analysis of docking results

AutoDock Tool 1.5.6. used for analyzing binding energies. Interactions between protein-ligand complexes and amino acids located in binding pocket analyzed using protein ligand interaction profiler, LigPlot used to get 2D imaged related to interactions and 3D imaged with interactions obtained using pymol.

Results and Discussion

Ligands identification -The major phytochemicals from each five ingredients of herbs contained in 'Curry Powder', that were extracted to water using various extraction techniques were found based on literature reviews. Those phytochemicals were again filtered into 21 ligands based on their percentage amount extracted to water from each five ingredients and with their drug-likeness properties.

Protein preparation - The protein sequence of the P domain from norovirus strain saga4 was submitted to SWISS-Model, an online modeling server, to discover the template with the highest GMQE, sequence similarity, and sequence coverage. The template obtained from the SWISS-Model with 0.81 GMQE was matched with the highest percentage sequence similarity PDB ID obtained from protein blast. After modeled protein was built, the Refinement was done by Galaxy Refine in the GalaxyWeb server. Then it provides five models, model with the highest RMSD was selected.

Table 1. Docking results

Ingredients of curry powder	Phytochemicals	Binding Energy (kcal/mol)
Fennel	Anethole	-4.83
	Fenchone	-6.31
	Limonene	-5.41
	Quercetin	-4.99
Curry leaves	Mahanimbine	-7.07
	Mahanine	-6.57
	Murrayanol	-5.96

Coriander	Alpha_pinene	-5.97
	Alpha-terpinene	-5 .52
	Camphor	-6.62
	Geraniol	-5.39
	Limonene	-5.33
Cinnamon	Alpha-phelendrene	-5.52
	alpha-terpinene	-5.52
	beta-phellendrene	-5.57
	Limonene	-5.41
Cumin	2-allylphenol	-5.01
	alpha_pinene	-5.97
	Cuminaldehyde	-5.55
	p-menthatriene	-5.29
	Terpinolene	-5.47

In this study molecular docking can be defined as a molecular modeling technique that can be used to predict the interactions between a large molecule such as protein (enzyme), nucleic acid and a small molecule (ligand). Knowledge of the preferred orientation may be used to predict the strength of association or binding affinity between protein and the ligand. Phytochemicals found based on literature reviews. An indication of the binding affinities of ligands relative to each other can be derived by analyzing and comparing binding energies. The higher negative free binding energy, the greater the chance of interaction with the protein. In this docking study rigid docking was performed. The internal geometry

of both the receptor and the ligand was considered as stiff in rigid docking. The binding energies for each protein-ligand complex retrieved using AutoDock 4.2 and they are shown in above table.

Above docking results obtained for site specific docking of P domain from norovirus strain saga4 in complex with HBGA type A and grid box was drawn to that site. Twenty-one ligands were docked to active site in P domain from norovirus strain saga4 in complex with HBGA type A receptor and out of that 6 ligands binding energy were greater than -5.5 kcal/mol. According to the results, higher negative binding energies reveal stable protein-ligand complexes. However, it should be noted that binding energy alone cannot predict the effect to the target protein function due to ligand-protein interaction. The binding pocket's location, non-covalent interactions, hydrogen bond interactions, salt bridges and their types of amino acids are involved for above mentioned interactions. To better understand the relationships between complexes that showed the greatest binding energies. Amino acids involving interactions with the ligands are depicted in above table (Table.1) for ligands giving binding energy greater than -5.5 kcal/mol.

Conclusions and Recommendations

In this study, the In silico studies were done only for few ligands that were taken from literature reviews which were the most abundant of each ingredient of curry powder. However, it should determine inhibitory activity for NoVs of other ligands that are not used here. Especially it should focus on water extracted ligands. This could be done by results obtained for ligands giving binding energy greater than -6.00 kcal/mol that identified in compound level by LC-MS/MS, LC-MS and further confirm using phytochemical screening. Also, more docking studies should be carried out with other human target antigens of NoV strain GII-4 and other dominant strains of NoVs such as (GI-1). Also, Further Molecular dynamics studies should be done in future to support the results of the present study.

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FOCUS AREA

Environment

ASSESSMENT OF THE ECOLOGICAL STATUS OF THE DIYAWANNAWA WETLAND BY AQUATIC MACROPHYTE-BASED LIMNOLOGICAL CONDITION INDEX

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Introduction

Aquatic macrophytes are key components in aquatic ecosystems. They are very important in maintaining the ecological balance in the wetlands and associated aquatic ecosystems [1]. Aquatic macrophytes also act as bio-indicators for monitoring water quality and ecological changes in wetlands and aquatic ecosystems [2]. Submerged aquatic macrophytes are accepted as the most common indicators of euthrophication because they have been proven vulnerable to changes in water quality. The absence of aquatic macrophytes may indicate water quality problems. The overabundance of aquatic macrophytes can result due to high nutrient levels and affect ecosystem health. They can serve as measurable indicators of the ecological conditions of surface water. Aquatic macrophyte-based monitoring methods can be applied to assess the health of wetland ecosystems. Further, applications of ecological assessment methods are important for the classification of the wetlands. This study was conducted to assess the ecological status of the Diyawannawa wetland by using the aquatic macrophyte-based Limnological condition Index, Water Quality Index and aquatic macrophyte-based diversity indices. Diyawannawa wetland ecosystem is an urban wetland system in Sri Lanka. Which is identified as an important marshland in the area and a wetland system that is at high level of risk.

Materials and Methods

Study area

Six study sites S1, S2, S3, S4, S5 and S6 were selected from the Diyawannawa wetland ecosystem. These sites included both rehabilitated area and non-rehabilitated areas of the Diyawannawa wetland ecosystem. S1, S2, and S3 sites were located in the non-rehabilitated area and S4, S5 and S6 sites were located in the rehabilitated area in the Diyawannawa wetland ecosystem.

Water quality parameters

From each site, surface water samples were collected in seven replicates. Water pH, conductivity, Total Dissolved Solids (TDS), water temperature and salinity were measured in situ at each site using a calibrated multi-parameter (YSI

Environmental Model-556 MPS). Dissolved Oxygen (DO) was measured using a DO meter (HQ 40b model-Hach) and water visibility was measured using a secchi disk in situ. In the laboratory, the biological oxygen demand 5 days after incubation (BOD_5), total phosphorus concentrations, Chemical oxygen Demand (COD), chlorophyll-a concentration and nitrate concentration were measured by following the standard procedure which is described by APHA (1992). The water quality data were used to calculate the water quality index for each site.

Aquatic macrophyte abundance

Line transect sampling method was followed to sample the aquatic macrophytes and they were identified by following the photographic guide of aquatic plants prepared by the National Aquaculture Development Authority [3]. The abundance of each aquatic species, at each site was recorded. These aquatic macrophyte abundance data were used to calculate the pollution index, biotic index (BI), aquatic macrophyte based limnological condition index (LICOI), Shannon Weiner diversity index, and Pielou's evenness index for each site.

Statistical Analysis

Statistical analysis was conducted using Minitab statistical software (version 17.0). The mean and the standard deviation of replicates were obtained using descriptive statistics for all the physicochemical water quality parameters and aquatic macrophytes abundance. One-way ANOVA followed by Tukey's pairwise comparison was used to assess the spatial variation of water quality parameters and the aquatic macrophytes abundance by after confirming the normal distribution using the Anderson Darling test.

Results and Discussion

There were no significant spatial variations in water pH, visibility, temperature, total phosphate concentration (TP) dissolved oxygen concentration (DO), salinity between the six sites ($P>0.05$). Significantly high conductivity, TDS and significantly low BOD_5 and COD were recorded from Site S1 compared to other sites ($p<0.05$). water quality of the studied sites is influenced by shading effects, agricultural activities, and industrial pollutions. Ten species of aquatic macrophytes were identified. They were *Eichhornia crassipes, Nymphaea ampla*, *Hydrilla verticillata, Cryptocoryne wendtii, Annona glabra, Pistia stratiotes, Ceratophyllum demersum, Nymphaea rubra,* and *Cypreus iria, Salvinia molesta*. There was no significance difference of the aquatic macrophytes abundance between the studied sites ($P>0.05$). Significantly high abundance of *Salvinia molesta, Cypreus iria, Nymphaea rubra, Annona glabra,* and *Eichhornia crassipes* were recorded from the non- rehabilitated area compaired to the sites in the rehabilitated area while the significantly high abundance of *Ceratophyllum demersu* and *Pistia stratiotes* were recorded from the sites in the rehabilitated area. However, low aquatic macrophytes abundance were recorded in the sites

with rehabilitated area due to anthropogenic pressure. Further wetland management programmes involve to manage the rehabilitated area in the Diyawannawa wetland.

The Shannon Wiener diversity index of the study sites ranged from 0.74 to 1.72. The highest Shannon Wiener diversity index was recorded from site S3 (1.72). The lowest Shannon Wiener diversity index was recorded at S1 (0.74). The Pielou's evenness index of the study sites ranged from 0.67 to 0.91. The highest pielou's evenness index was recorded from S1 While the lowest pielou's evenness index was value was recorded from S4. The pollution index, percentage biotic index, limnological condition index and water quality index for each site is given in Table 1. The pollution index of the study sites ranged from 13.77 to 35.22. The percentage biotic index of the study sites ranged from 76.92 to 94.1. The limnological condition index of the study sites ranged from 5.9 to 23.71. The water quality index of the study sites ranged from 5.79 to 39.19.

Table 1. The Pollution index, percentage biotic index, limnological condition index, and water quality index for each site the Diyawannawa wetland.

Site	Pollution index	Percentage Biotic index	Limnological condition index	Water quality index
S1	13.77	76.92	23.08	5.79
S2	23.71	78.77	21.23	29.20
S3	23.02	91.11	8.89	28.06
S4	29.68	80.04	19.96	34.28
S5	31.05	80.31	19.69	26.64
S6	35.22	94.10	5.90	39.19

Based on the calculated limnological index value, wetlands can be categorized in to four categories. (LICOI ≥ 35: slightly contaminated; 17≤ LICOI < 35: moderately polluted; 4≤ LICOI < 17: heavily contaminated; LICOI < 4: Severely contaminated) [4]. S1 of the non- rehabilitated area showed highest limnoloical condition index value (23.08). According to the calculated percentage BI and LICOI value, this site can be catergorized as moderately polluted site. S6 of the rehabilitated area showed lowest limnoloical condition index value (5.90). This site can be catergorized as heavily contaminated site. A decline in an aquatic macrophyte community may indicate water quality problems as well as changes in the ecological status of the natural water body. Further, the presence of aquatic macrophytes may enhance water quality because they can absorb excessive loads of nutrients. Aquatic ecosystem properties including water transparency and nutrient transparency can be changed due to changes in the composition of aquatic plants. Rehabilitated area is regularly subjected to management actions. Invasive alien aquatic plants are removed under that management programme. This may have resulted in presence of highly contaminated sites in the

rehabilitated area. Further, the water quality of the wetlands can be categorized into five groups based on the calculated WQI value (0<WQI≤25: Very bad. 25<WQI≤50: Bad; 50<WQI≤75: Medium; 75<WQI≤100: Good. WQI>100: Excellent) [5].

According to the WQI-based classification, in the present study, the water quality of the site S1 was categorized as medium and the water quality of sites S2, S3, S4, S5, and S6 can be expressed as bad. Therefore, the overall water quality of this wetland can be considered as unsuitable for drinking and for sustaining healthy aquatic life.

Conclusions and Recommendations

According to the calculated percentage BI and LICOI values sites S1, S2, S4, and S5 of the Diyawannawa wetland can be categorized as moderately polluted sites with acceptable limnological conditions.

Results of the present study revealed that the LICOI can be used as an important tool for monitoring the water quality trends in this wetland ecosystem. However, it is recommended to assess the usability of LICOI in other aquatic environments based on the biological parameters of the respective ecosystems.

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REDUCTION OF WATER LEACHING POTENTIAL IN SANDY LOAM SOIL BY USING BIOCHAR

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Introduction

A healthy soil is the cornerstone of a healthy food system, and two essential elements of healthy soil are nutrients (N, P, and K) and soil organic carbon. Due to soil erosion or leaching, tropical ecosystems are particularly vulnerable to nitrogen loss [1]. In tropical and subtropical areas, heavy rainfall causes N, P, and K to leach from soil environment. Nutrient and minerals leaching has the potential to degrade soil fertility, hasten acidification, raise fertilizer expenses for farmers, and decrease total harvests and products [2]. N and P leaching, as well as agricultural runoff, are major contributors to non-point source contamination, which harms drinking and ground water. Agricultural runoff contains more pollutants and nutrients, which contribute to eutrophication and biological oxygen demand [3]. Thus, innovative and effective agricultural management strategies must be found, tested, and monitored in order to lessen agriculture's impacts on the sustainability of water and soil resources. A solid bioresource called biochar is created by pyrolyzing organic waste. Remains from agriculture and forestry can be used as a source material to create high-quality biochar [4]. As a result, adding a porous substance with a high carbon content like biochar to agricultural soil has become a practical method for enhancing the soil's ability to retain water, its quality, the stability of its organic matter, and its ability to retain nutrients [4]. This suggests that biochar could help soils to retain more water, which should increase agricultural output in dryland areas that aren't watered and decrease the amount of irrigation water needed to grow crops [5]. Applying biochar to the soil could increase crop output and soil fertility by reducing leaching and possibly supplying nutrients. It has been discovered, nonetheless, that the effects of biochar on nutrient leaching and organic carbon retention vary depending on the applied biochar's pyrolysis temperature, raw material, and soil type [1].

In this research, sandy loam soil samples were taken from a tropical area and treated for 90 days with two types of wood dust biochar that had been pyrolyzed at 450 and 550 degrees Celsius. The purpose of this study was to determine how

the application of biochar affected soil water retention and leaching. The results are meant to be helpful in figuring out biochar's potential for soil retention.

Materials and Methods

Soil samples and biochar preparation

Soil samples were collected at a depth of 0-20 cm from the Kaliodai river, which is located in the east of Sri Lanka (7017'51.12" N latitude- 810 50' 54.12" E longitude). The soils from the experimental site were sandy loams. The soil samples were kept at room temperature after being air dried and sieved through a 0.85 mm screen. Wood sawdust from the Pandiruppu sawmill was used as the feedstock for the biochar used in the study. To reduce the amount of free moisture that would have an impact on how well the pyrolysis reaction works, the biomass was dried in a drying oven (WOF-155, Witeg, Germany) for 24 hours at 105 °C. The biomass was then sieved using 0.85 mm sieve. A slow pyrolysis technique was used to make the biochars in a muffle furnace in the engineering laboratory at the Faculty of Technology. Air dried sawdust was tightly packed in a metal container 0.15 m in diameter and 0.45 m in height, with a hole (0.01 m in diameter) at the top. The container was closed with a lid and placed in the muffle furnace (MF1400-30). B-450 and B-550, two biochar materials made through pyrolysis at 450 and 550 °C for two and one hour, respectively, were tested.

Column Leaching Experiment

Leach columns were constructed using polyvinyl chloride tubuing (5.4 cm internal diameter and 42 cm height) and a wooden table. The bottoms of the tubes were tightly covered with muslin cloth. To maintain a constant soil bulk density, the biochar materials were well mixed with the collected sandy loam soil at application rates of 0 (T1), 1.25% (T2), 2.5% (T3), and 5% (T4) (w/w). The treatments tested in this study consisted of two biochar (B-450, B-550) with four replicates and four application rates. Soil columns were placed vertically on a wooden table and incubated for two months with the addition of a small amount (50 ml) of water. Two months after the incubation, distilled water was added to the soil in the columns up to their saturation point. Then, 200 ml of distilled water was added to each leach column once a month and leachates were collected after two days.

Figure 1: Column leaching Experiment

Statistical Analysis

Using IBM SPSS Statistics 16 for Windows, data were examined. A two-way analysis of variance was used to perform a mean separation analysis of the Tukey Post Hoc test on the data sets, with a significance level of 0.05.

Results and Discussion

The characteristics of the soil samples and biochar materials are displayed in Tables 1 and 2. The studied soil had a sandy loam texture, a lower pH, and a higher OC content. B-450 and B-550 (wood sawdust pyrolyzed at 450 and 550 ^{o}C) had pH values of 6.9 (neutral) and 8.2 (alkaline), respectively.

Table 1. Properties of the Soil

Properties of soil		**Values**
Soil texture	Sand %	68.1
	Silt %	16.2
	Clay %	15.7
	Sandy loam	
Soil colour	Dry condition	2.5Y3/2 Dark grayish brown
	Wet condition	2.5Y3/2 Very dark grayish brown
Bulk density		1.45g/cm^3
True density		2.56 g/ cm^3 Repeat the testcorrect
Soil pH		5.65
EC (µS/cm)		80
Organic matter content		0.54%
Cu (ppm)		3.5
Porosity & PD		43.4%
Water Holding Capacity		52%
Moisture content		20%
Total Nitrogen		0.17%
Available Phosphorus		28 ppm

Exchangeable K as Ammonium acetate extract (meq/100g)		0.54
CEC		4.77 cmol kg–1
Cd		0.5 mg/kg

Table 2. Properties of biochar

Properties	Biochar	Values
pH	B-450	6.9
	B-550	8.2
EC	B-450	3.43 dS/m
	B-550	2.12 dS/m
Yield%	B-450	40
	B-550	35
Ash%	B-450	14.4
	B-550	16.0
Organic Carbon%	B-450	23.4 %
	B-550	27.3 %
Color	B-450	Grayish Black
	B-550	Dark Black

Table 3. Mean volume of the leachate from soil column

	Treatments	Mean Volume of Leachate			Water retaining capacity		
		August	September	October	August %	September %	October %
B-450	T1	95.00	118.00	101.00	55	51	42.5
	T2	63.75	82.50	73.75	67.5	51	57
	T3	42.00	60.25	50.00	80.5	62.5	69.5
	T4	35.50	42.50	42.50	86.5	83	83
B-550	T1	97.50	68.25	70.00	30	65.5	66.5
	T2	51.00	62.50	54.00	75	69.5	70.5
	T3	40.00	49.75	41.25	81.5	78	77.5
	T4	25.75	41.00	25.00	89.5	80.05	85

For each flushing event, both biochar-treated samples showed considerably lower leachate quantities than the control (Table 3). As a result, samples treated with biochar retained more water than the control.

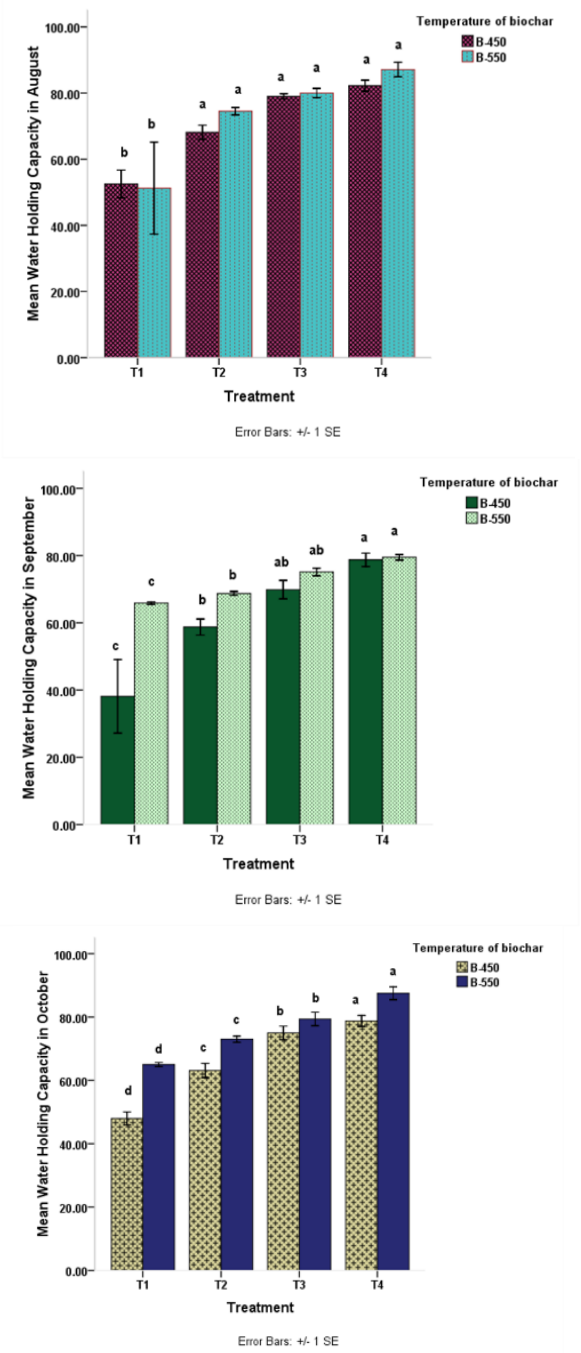

Figure 2. Comparison of mean Water Holding Capacity (in August, September & October in two different biochar

Table 4. The average monthly water leachate of each treatment with two different biochar applications

Month of leachate collection	Temperature of biochar	Treatments	Mean	Std. Deviation
August	B-450	T1	95.0000	16.83251
		T2	63.7500	8.53913
		T3	42.0000	3.16228
		T4	35.5000	6.60808
	B-550	T1	97.5000	55.60276
		T2	51.0000	4.54606
		T3	40.0000	5.71548
		T4	25.7500	8.77021
September	B-450	T1	104.00	43.76738
		T2	82.5000	9.53939
		T3	60.2500	11.02648
		T4	42.5000	7.93725
	B-550	T1	68.2500	1.50000
		T2	62.5000	2.38048
		T3	49.7500	4.57347
		T4	41.0000	3.36650
October	B-450	T1	1.0400E2	8.20569
		T2	73.7500	8.95824
		T3	50.0000	8.60233
		T4	42.5000	7.00000
	B-550	T1	70.0000	2.58199
		T2	54.0000	3.91578
		T3	41.2500	8.53913
		T4	25.0000	8.08290

A highly significant influence on leachate was shown by the treatment every month. The effect of temperature on treatment was highly significant only in September and October leachate collection. The significant influence of temperature on leachate was observed in September and October only (Table 5). In each flushing cycle, both biochar-treated samples recorded significantly lower leachate quantities than control (Figure 1, Table 3). This indicates that, biochar treated samples retained about more water than the control. Among the treatments leachate content is very low in the treatment T4 which may be due to higher water retention by the treated soil. The biochar-treated samples continued to significantly retain more water in the soil columns each month, even though the variations in leachate volume between the control and biochar-treated samples in this study gradually declined (Table 4). These findings showed that biochar materials, especially B-550, had a significant capacity to preserve water in the soil samples when used at a rate of 5% (Figure 1). This might be because biochar was applied to the soil, which has a highwater retention capacity. One of the results demonstrated that soil water, DOC, N, P, and K leaching could be successfully reduced using both biochar produced at 300 and 600 degrees Celsius [1]. After incubation, it was observed that the biochar-treated soil had lower leachate volume and nutrient content than the control soil [1]. In a sandy loam soil, using biochar could increase soil and water conservation by enhancing

soil structure, boosting infiltration rates, and reducing runoff water and soil erosion [1].

Based on two facts (1) a high level of vaporization (roughly 30°C) for the soil column after water addition, and (2) the volume of adding water (200 ml to the soil column each month) still did not match the pore volume of the soil column—it was assumed that the biochar treatments could still hold water after adding water. Increased water retention may help stop leaching of nutrients. Biochar materials also have a deterrent impact on the mineralization of organic N in terms of the physical preservation of organic matter [5]. By minimizing the leaching of more water and nutrients from soils, it may be possible to protect nearby surface water bodies and groundwater from contamination. This study indicates that biochar can favor both soil and water conservation. Further studies are needed to see whether these advantageous impacts could spread to the field and to water bodies downstream.

Conclusions and Recommendations

According to the results, incorporating wood sawdust biochar into sandy loam soil samples could improve soil health by increasing the soil's capacity to hold nutrients and reduce water evaporation. Both types of biochar effectively helped to conserve water in soil varied depending on pyrolysis temperatures. Future research should examine the potential impact of biochar application on eutrophication and pollution in subsurface and surface water bodies.

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MITIGATION OF HYPEREUTROPHIC STATUS IN BEIRA LAKE WITH COCONUT SHELL BIOCHAR

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Introduction

The Beira Lake is the largest water body in Colombo city. In today water body in the lake is extremely contaminated due to reclamation of the sections of the Beira for commercial development and use of it for discharge urban and industrial wastewater [1]**.** Phosphorus and nitrogen are the key nutrients that identify as leads to eutrophication. The sources of those nutrients can be point or nonpoint such as rainfall, runoff, industrial and municipal wastewater [2]. Also, the accumulation of sediment nutrients is a major problem because it acts as a nutrient source. Sediment takes up the external phosphorus load and releases adsorbed phosphorus to water when the reduction of external input [3]. These eutrophication and anaerobic conditions of water significantly reduce the financial and aesthetic value of water bodies, emitted toxic gasses which can directly affect the health and make a pungent odor [4]. Restoration of eutrophic lake ecosystems has often focused on controlling phosphorus inputs. These research efforts introduce restoration methods that target the controlling of Phosphorus from lake sediment through the sediment water column experiment set up by using a key controlling substance of biochar.

Materials and Methods

Preparation of Experimental Setup

The experiment setup was made by using 110 mm diameter PVC pipe and obtaining a height of 1000mm. Biochar was produced by using coconut shells. After that coconut shells were slowly pyrolyzed at 500 °C with 300 °C/hr heating flow rate. Finally, the resultant biochar was crushed and sieved through 10mm and 5 mm mesh sieves.

Area of Study

The practical work pertaining to the aims and objectives was carried out in the Beira Lake on the 30th of December 2021 from 09:30 a.m. to 12:30 p.m. The two-sampling locations were selected from Gangarama Lake.

Sample Collection

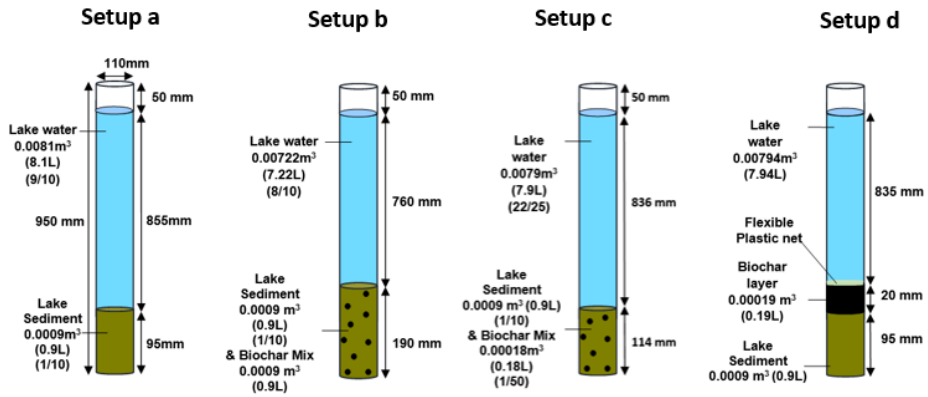

Figure 1. Experimental setup

The lake sediment samples were placed in the bottom of the experimental setup as of figure 1. The lake water was overlaid on the top of sediments in the experimental setup. The mix proportions shown in Table 1 were used for the 07 experimental setups

Table 1. Mix proportions of lake water, sediment and Biochar used in the experimental setups.

Experiment No	Lake water (L)	Sediment (L)	Biochar Mix (BM)/Biochar Layer (BL)
a	8.1	0.9	-
b	7.22	0.9	BM/0.9
c	7.9	0.9	BM/0.18
d	7.94	0.9	BL/0.19

Results and Discussion

Analysis of Lake Water Nutrient Concentration in The Experimental Setup

Figure 2 shows the variation of nutrients concentrations over the time in experiment setup water columns. The phosphate concentration was rapidly increased during the first 07 days in the first setup. However, these changes can be contributed to the disturbance by sampling. Nitrate and Nitrite concentration gradually decreased in the first three weeks. Nitrate concentration was rapidly increased after three weeks (figure 3) and it can be understood that one of the reasons for drastic increase of nitrate concentration is due to the oxidation of nitrite to nitrate. The ammonium concentration clearly decreases with time. It

can be understood that negatively charged biochar surfaces balance with positively charged cations in aqueous environments.

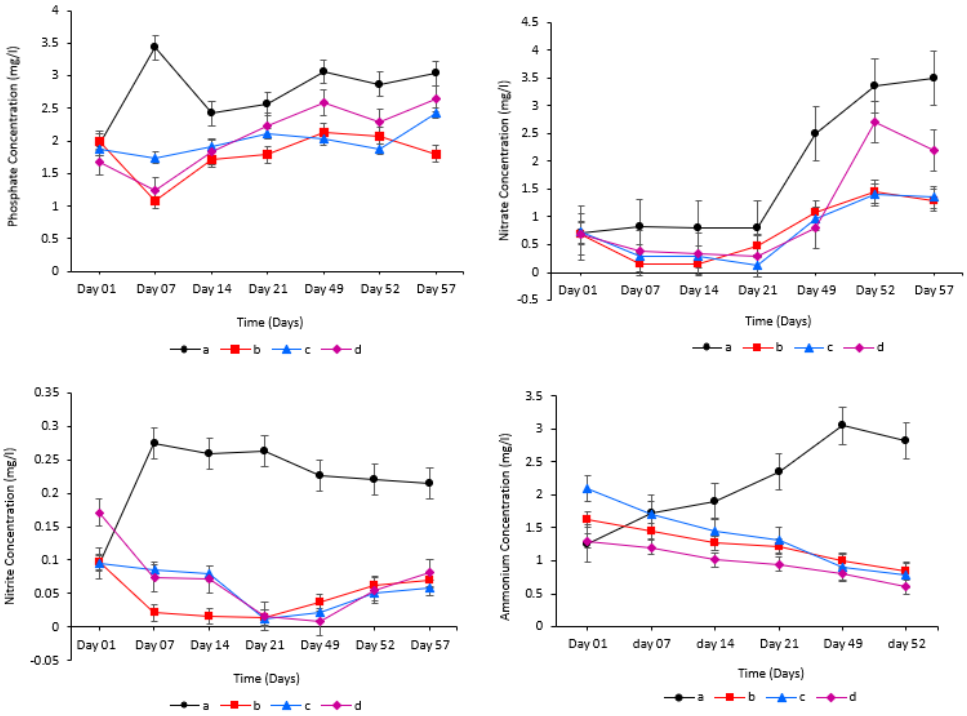

Figure 2. Variation of nutrient concentration with time

Analysis of Lake Sediment Nutrient Concentration in The Experimental Setup

According to the above graphs it can be concluded that all setups which are treated with biochar show significant reduction of nutrients in the lake sediments. It can be identified biochar mixed setups were shown more than 50% of nutrient reduction from sediments because of biochar has a relative structure to carbon matrix with a high degree of porosity, presence of alkaline cations, with high specific surface area, open-pore structure, and good adsorbent capacity for the removal of various contaminants. Nutrient reduction in first setup can be due to the release of the nutrients from sediment to the overlaid water.

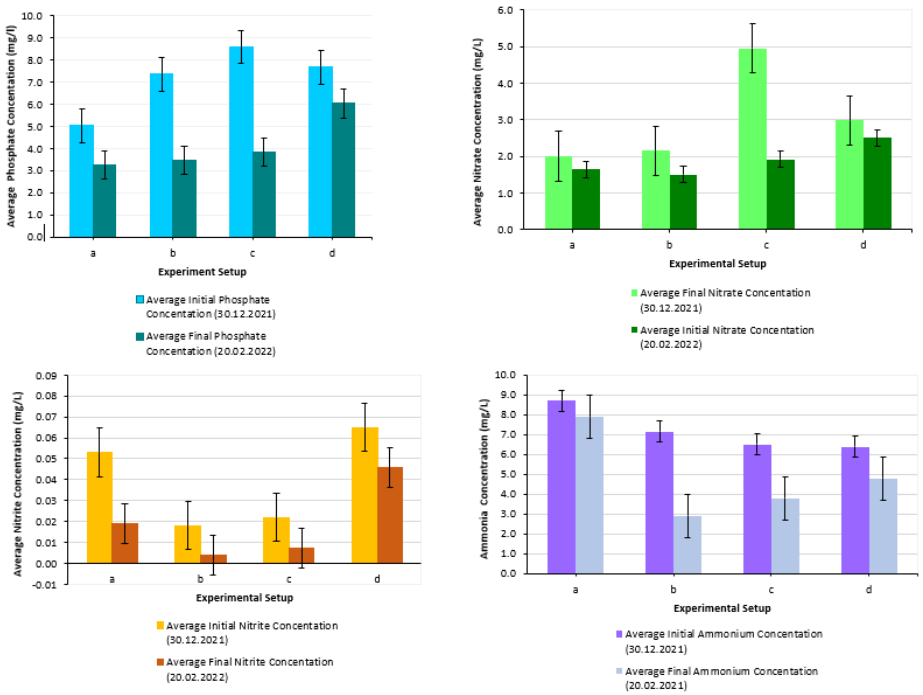

Figure 3. Variation of initial & final nutrient concentration with setup

Conclusions and Recommendations

In this investigation lake sediment acts as a nutrient source for the eutrophication and releasing nutrient from polluted sediment in Beira Lake (Gangarama Lake) was studied in a laboratory scale. Also, removal of nitrogen and phosphorus from water via adsorption by unmodified biochar has been studied. Biochar exhibits a high efficiency for treating polluted sediments and the low-cost of biochar provides an advantage as a remediate material to adsorb and degrade pollutants, and biochar could perform comprehensive functions in sediment remediation. According to the laboratory experiments biochar mix with sediment is more efficient way to remove nutrients than overlaid biochar layer on sediment surface. Suggested that most of the unmodified biochar only do not show long-lasting efficiency in absorbing Nitrogen and Phosphorus In water. Therefore, modification is necessary to significantly enhance the adsorption capability of biochar for Nitrogen and Phosphorous removal.

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Dioscrea alata L.YAM EXTRACT AS A NATURAL ACID-BASE INDICATOR

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Introduction

Over the years, synthetic indicators have been the choice of acid-base titrations. Synthetic indicators have certain disadvantages such as high cost, chemical pollution, and toxic effect on users such as diarrhea, eye irritation, skin irritation, respiratory track irritation and skin rashes [1]. Methyl orange is a water soluble acid-base indicator containing highly toxic, carcinogenic and teratogenic aromatic and –N=N- groups. Methyl orange containing waste water released to the environment can lead to deterioration of water quality and it requires costly treatment methods to remove the indicator from waste water before releasing into the natural environment. Therefore, searching alternative acid-base indicators of natural origin is a timely need. Several studies by various investigators have reported the effectiveness of acid base indicators of natural origin; flowers such as *Rosa setigera, Hibiscus Rosa sinensis* [1], *Catharanthus rosea, Nerium oleander* [2], leaves such as red cabbage, fruits such as *Dioscorea bulbifera* L. [3] for titrimetric analysis. Natural indicators would be cheaper, more abundant, safer, less toxic, easier to extract and more environmentally friendly than the synthetic indictors [1]. This study evaluates and validates the acid-base indicator properties of *Dioscorea alata* L. yam extract and it encourages to takes maximum advantage of what nature can offer in a sustainable way.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents used were of analytical grade.

Plant material

Fresh *Dioscorea alata* L. yam was purchased from a local market in Giriulla, Srilanka.

Preparation of Dioscorea alata L. *yam powder*

The preparation of *D. alata* yam flour was carried out according to the method done before with slight modifications [4]. The yam tubers were cleared from any dirt. Then it was peeled and rinsed with water and cut into slices (2 cm thickness). The sliced sample was steamed blanched in steamer for 10 minutes at the temperature of 95 ℃. Blanched sample was sun dried for 3 days. Dried yam chips

were ground with a blender in order to obtain yam flour. The flour was stored in a brown bottle with desiccant gel and kept at 4℃ in a refrigerator.

Preparation of Dioscorea alata L. *extract*
Yam powder (10 g) was extracted with 150 mL of acidified methanol solution at 60℃ in a water bath with occasional shaking for 10 minutes. The obtained extract was filtered and centrifuged at 8000 rpm for 15 minutes. The supernatant was kept at 4 ℃ in a brown color bottle for further analysis.

Measurement of the maximum absorption wavelength of D. alata yam pigment
The pH of the extract was measured using a pH meter. The extract was scanned from 400 nm to 700 nm using UV-Vis spectrophotometer to determine the maximum absorbance of *D. alata* pigments.

Preliminary phytochemical screening
D. alata extract was tested for presence of alkaloids, flavonoids, anthocyanidins, tannins and saponins using a previously reported method [5].
Acid- base titrations using D. alata extract as the indicator
Four types of acid-base titrations were carried out at room temperature using *D. alata* extract (1 mL) as the indicator. The four types of titrations were again performed using Phenolphthalein, Methyl red and Methyl orange. All titrations were repeated 3 times to check the precision.

Development of acid-base test strip.
The Whatman filter papers (12.5 cm, 23 µm pore size) were soaked in the extract for 10 minutes and air dried. Then dried papers were cut into strips. Paper strips were stored in a bottle and kept in dark place.

Results and Discussion
The extract of *Dioscorea alata* L. has a pH of 3.5 and red in color. The visible light absorption spectrum (Figure.1) of the extract has prominent peak (λ_{max}) in the 500-550 nm wavelength regions. It confirms the presence of anthocyanidin [2]. Preliminary phytochemical screening also confirms the presence of anthocyanidin (Table 1).

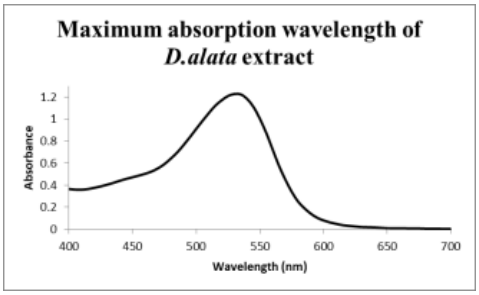

Figure 1. Visible spectrum of *Dioscorea alata* L. extract

Table 1. Preliminary Phytochemical screening of acidified methanol extract of *Dioscorea alata* L.

Group of phytochemical	**Present (+)/ Not present (-)**
Alkaloids	-
Flavonoids	+
Anthocyanidin	+
Tannins	+
Saponins	+

Anthocyanins are colored water soluble pigments which have excellent pH responsive properties Figure 2.The UV-Vis absorption spectra of the extract at different pH values are shown in Figure 3.The color variation of the yam extract is mainly due to the structural transformation of anthocyanin present in the extract [1] .The color variation of pH test strips immersed in different pH solutions is shown in Figure 4.Its initial color, red remains unchanged in acidic media (pH<7) and color red changed from red to blue in basic media (pH>7). The applicability of the *D. alata* extract as an acid-base indicator in strong acid-strong base, strong acid-weak base, strong base-weak acid, weak acid-weak base was determined by comparing the end points obtained with those of methyl orange, methyl red and phenolphthalein Table 2.

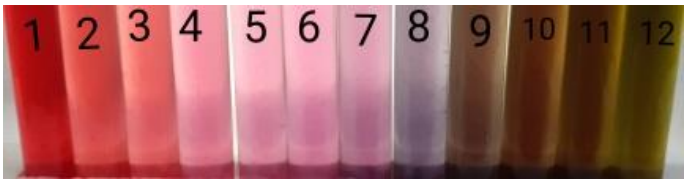

Figure 2. Color variation of *D.alata* extract from pH 1.0-4.0 red, pH 5.0-6.0 pink, pH 7.0 purple, pH 8.0 blue, pH 9.0 bluish-violet, pH 10.0 -11.0 brown, pH 12.0 yellow

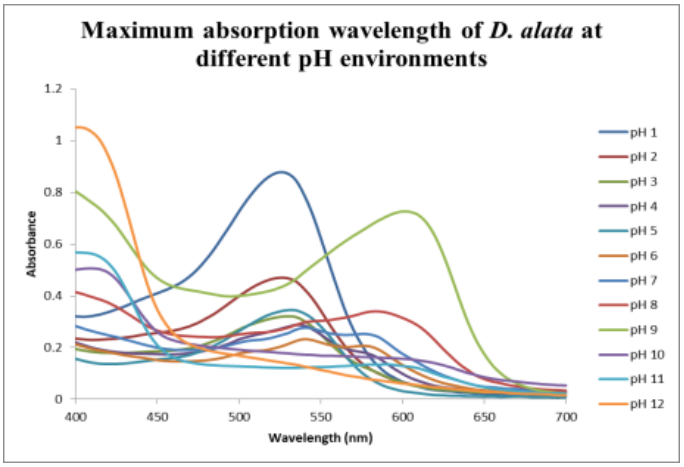

Figure 3. Visible spectrum of *D.alata* extract at different pH (1 to 12)

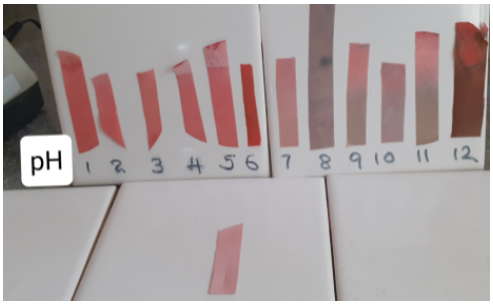

Figure 4. Color variation of acid-base test paper strips of *D.alata* extract at different pH

Table 2. The end point and color change at the end point in titrations using standard acid-base indicators and *D. alata* extract

Titrant/Titrand	Indicator	Volume of titrant in (mL)*	Color change
NaOH/HCl (10.0 mL)	Phenolphthalein	9.00±0.05	Colorless to pink
	Methyl orange	9.10±0.05	Red to orange
	Methyl red	9.00±0.05	Red to yellow
	D. alata extract	9.10±0.05	Red to blue
HCl/Na_2CO_3(10.0 mL)	Methyl orange	25.40±0.1	Yellow to red
	Methyl red	25.10±0.05	Red to yellow
	D. alata extract	25.10±0.1	Blue to Red
$NaOH/CH_3COOH$ (10.0 mL)	Phenolphthalein	10.40±0.05	Colorless to Pink
	Methyl red	9.70±0.05	Red to Yellow
	D. alata extract	10.40±0.05	Red to blue
CH_3COOH /Na_2CO_3 (5.0 mL)	Phenolphthalein	6.60±0.1	Colorless to pink
	Methyl red	5.40±0.05	Red to Yellow
	D. alata extract	6.00±0.05	Red to blue

**Data represented as mean ±SE (n=3) [NaOH]=0.1 M [HCl]=0.1M, [CH_3COOH] =0.1M, [Na_2CO_3] =0.1M*

The equivalence point of the all titrations using the *D. alata* extract as indicator is similar to the equivalence point of standard indicators. The results obtain show that the routinely used chemical indicators can be replaced by *D. alata* extract which is performed well in all four titrations with sharp and clear color changes.

Conclusions and Recommendations

The study revealed that the extract of *Dioscorea alata* L. can be used as a substitute for synthetic acid-base indicators and it is more advantageous due to low cost, safety, availability, easy and simple extraction procedure, excellent performance with accurate and precise results.

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ADSORPTIVE REMOVAL OF OIL SPILLS BY WASTE-DERIVED DENDRO BIOCHAR: A GREEN ORIENTED APPROACH

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Introduction

Oil spills are one of the main ways that hydrocarbons are released into aquatic or terrestrial environments. These large spills had a considerable negative impact on ecosystems. Sea birds, other marine wildlife and mangrove were severely impacted by the majority of oil spills that took place in the marine environment [1]. Oil spills along the shoreline posed a significant threat to the livelihoods of people and the life of other living creatures. These hydrocarbons have the potential to contaminate foods, enter the food chain, and have a significant negative effect on community health.

Sri Lanka is a closely located island to the Indian Ocean shipping route with a few harbors in easily accessible locations. Sri Lanka has a history of oil spills along its shorelines and in the ocean. Several events in the maritime environment have occurred since 2010, including an oil spill in the Lunawa lagoon in Thaldiyawatta in 2015, a furnace oil leak in Heen Ela, Dematagoda, in 2018, and the new diamond fire incident in 2020 which is located offshore from Sangamankanda Point. The most significant spill occurred in 2021 and is known as the X-press Pearl cargo ship fire incident [2]. Such disasters left significant ecological damage that Sri Lanka would likely suffer for decades. Heavy fuel oils which have a high viscosity, are the major of numerous spills. Engine or motor oil is a kind of high viscous oil which is used in automobile service stations and due to transportation industry has grown, a large number of auto service stations have appeared. However, they have lack of proper cleanup procedures for their oily effluent discharged, which may contain a variety of pollutants like gasoline, detergents, shampoo, and used auto-mobile motor oils. These openly discharge into the environment and may contaminate water sources with hydrocarbons.

Numerous studies have already attempted to develop cleanup techniques for oil spills over time. The majority of remediation techniques fall into one of four categories: chemical, mechanical, biological, or physiochemical. Various investigations have focused on adsorption-based techniques, which have gained

popularity in part because they are readily available, affordable, non-flammable, and nontoxicity. As a sorbent substance, biochar is beneficial from every angle. Because of its great adsorption capabilities, such as its enormous surface area, porous structure, and hydrophobic properties. it is used to enhance soil, and remove heavy metals, and other pollutants. Many studies have been undertaken in recent years on the application of biochar for remediation approaches. Dendro biochar is a residue of dendro power plants and woody biochar derived from *Gliricidia sepium*, while there have been fewer or no studies on its use in oil removal procedures. This study looked into the viability of applying biochar derived from the Mahiyanganaya dendro plant (MBC) as a sorbent material for the oil removal process. The objective of this research is to examine the oil sorption capabilities of the dendro biochar derived from Mahiyanganaya dendro power industry.

Materials and Methods

Engine oil (Havoline SAE 15W-40) was used to simulate an artificial oil spill in laboratory studies. Sea water was collected from a Sarakkuwa beach, Sri Lanka and refrigerated (10 °C) to keep the quality from deteriorating. Biochar was obtained from a dendro power plant in the Mahiyanganaya area, which was formed as a waste byproduct of the gasification process that is utilized in Sri Lanka to generate electricity [3]. MBC that had been dried and crushed was sieved through a 1 mm mesh and used in subsequent studies. By mixing 1.5 g of oil with 25 mL of seawater (i.e., 60 g L^{-1}) in a 50 mL beaker, an artificial oil spill was created. Then MBC was added (10, 24, 50 g L^{-1}) on to the oil that was floating on oil. The oil-adsorbed biochar clump could be readily detached using a spatula and placed into the Buchner funnel lined with filter paper () for filtering any surplus oil and water by sucking down. To remove the adherent and adsorbed water, filter paper with oil-laden MBC was dried in an oven at 60 °C until reaching a consistent weight (2 h). Using mass balancing, dried filter papers were weighted to calculate the amount of adsorbed oil in the MBC [4].

Characterization

The moisture content, volatile matter, ash, and fixed matter of the MBC were calculated using proximate analysis. The moisture content of the biochar was calculated by heating MBC-containing opened crucibles at 105 °C for 24 hours until a constant weight. The volatile/mobile matter and ash content were estimated by heating MBC to 450 °C for 1 hour in a covered crucible and 750 °C for 1 hour in an opened crucible, respectively. By deducting the total percentage of moisture, mobile matter, and ash content, the fixed matter was calculated. Morphological analysis of MBC was carried out using a field emission scanning electron microscope (SU6600 FESEM, Hitachi Ltd, Tokyo, Japan). Spectral analysis was done using a Fourier Transmission Infrared Spectrometer

(FTIR; Thermo Scientific, Nicolet iS10 spectrometer, USA) to observe the differences in the chemical nature of MBC before and after oil adsorption.

Adsorption kinetics

Optimization of contact time for oil spill adsorption was investigated at 25 °C to understand the kinetic behavior and equilibrium time. The doses of biochar were maintained at 10, 24, 50 g L^{-1-} and oil concentration at 60 g L^{-1}. The samples were taken at appointed time intervals (10, 30, min, and also for 1, 2, 4, 6 h). The best sorbent-to-oil ratio (SOR) was accessed by testing three different sorbent-to-oil ratios (SORs, 5:6, 2.4:6, and 1:6) for kinetic adsorption at three different MBC doses (50, 24, and 10 g L^{-1}). Non-linear kinetic models, including pseudo-first-order and pseudo-second-order, were used to model the experimental kinetic data.

Adsorption Isotherm

The isotherm studies were conducted using the best dose (24 g L^{-1}) of MBC among the three SOR. The oil concentrations utilized to perform the adsorption isotherm ranged from 10 to 100 gL^{-1}, while other variables remained constant (MBC dose (24 g L^{-1}), at 25 °C). The experimental data were modelled using nonlinear models of Freundlich, Langmuir, and Sips isotherm models.

Results and Discussion

Characterization

The moisture content, mobile matter, ash content, and resident matter were found to be 9.81± 0.15%, 33.86± 0.21%, 15.47± 0.43%, and 40.87% respectively. It indicates that MBC has a high resident matter content, with carbon making up the majority of it.

Figure 1a shows SEM imaging of pristine biochar, which shows a porous surface. Observing images with uniformly distributed voids allows one to spot macro and micro holes (100 μm). In addition to chemical binding, this distribution of pores may stimulate the physical adsorption of oil into pores.

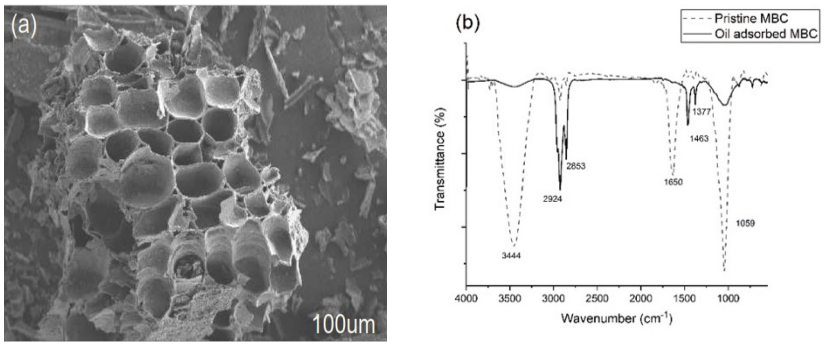

Figure 1. (a) SEM image (x500, 100 um), **(b)** FT-IR spectral analysis of pristine MBC and oil-adsorbed MBC

MBC was analyzed spectroscopically before and after oil adsorption using FT-IR. The clear contrast between Pristine and oil adsorbed MBC graphs, as illustrated in Figure 1b, was caused by hydrocarbon adsorption into the biochar. The stretching vibration of an O-H bond has a wide, broad peak in pristine MBC at 3444 cm^{-1}, whereas the stretching vibration of a C=C bond has a sharp, narrow peak at 1600–1670 cm^{-1}. The newly formed strong peaks at 2924–2853 cm^{-1} and 1377–1463 cm^{-1}, are associated with oil-adsorbed MBC that was related to C-H stretching and C-O/ C-H bending respectively [5]. This additional peak emergence is a sign that the MBC has absorbed hydrocarbons (engine oil).

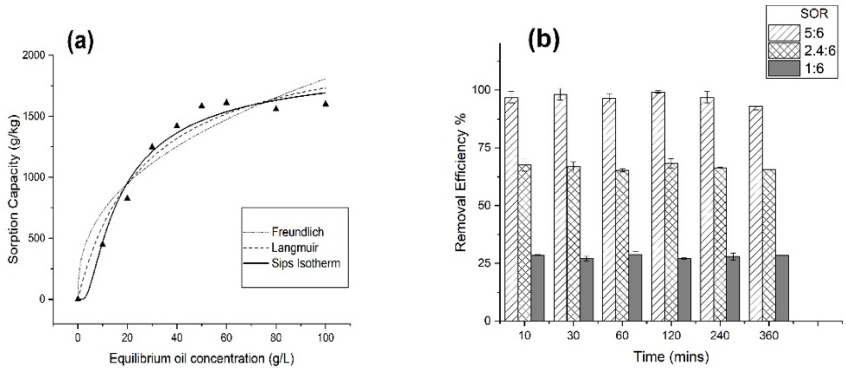

Figure 2. (a) Isotherm adsorption **(b)** Removal efficiency of three sorbent to oil ratios vs time

Adsorption Kinetics

To evaluate the adsorption kinetics, three SOR values were utilized. Oil uptake rates was fast at the initial stage and reached equilibrium within the first 15 min. Using a large dose of MBC is a waste of material since increased SOR (5:6) removed over 95% of the oil though relatively less adsorption capacity was found. The average adsorption capacities of MBC at equilibrium were 1170, 1650, and 1630 g kg^{-1} for sorbent-to-oil ratios of 5:6, 2.4:6, and 1:6, respectively. As a result,

when SOR was set to 2.4:6, relatively good adsorption performance was observed. As shown in Figure 2b, with SOR values falling from 5:6 to 1:6, removal efficiency decreased multiple times, from 95 to 30%. The pseudo-second order model among the kinetic models tested, provided excellent experimental data fitting (R^2 > 0.99).

Adsorption Isotherm

As per the Figure 2a the isotherm data did not fit the Langmuir and Freundlich models well, but the Sips isotherm model provided the best correlation coefficient (R^2 > 0.98) as shown in Table 1.

Table 1. Sips Isotherm model data of MBC (24 g L^{-1}) for engine oil adsorption at 25 °C and 1 h equilibrium

Isotherm Model	*Isotherm model parameters*	*Values*
Sips Isotherm	q_m(g kg^{-1})	1952 ± 104
	K (L g^{-1})	7.38 ± 0.49
	n	105.9 ± 7.09
	R^2	0.98

Conclusions and Recommendations

The cleanup of engine oil from oil-contaminated water was effectively accomplished using waste biochar generated at the Mahiyanganaya dendro power plant. The amount of biochar used has had a significant impact on the adsorption performance. According to sorption studies optimum sorption results were observed by the 2.4:6 sorbent to oil ratio which used 24 and 60 g L^{-1} of MBC and oil respectively. Oil uptake was fast and reached to equilibrium in less than 15 min. The distribution of pores and their morphological characteristics, which were examined by SEM, support the physical sorption of oil rather than chemical bindings. The change in chemical properties and the formation of strong peaks caused to hydrocarbons in spectral analysis (FT-IR) provided evidence for oil adsorption in MBC. Hence, this motivates researchers to investigate the use of dendro biochar, a waste byproduct of the dendro power industry, in the reduction of oil pollution. The further experiments will be carried out to compare the adsorption performance of biochar derived by several other dendro power industries.

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FOCUS AREA

Food, Nutrition and Agriculture

ECO-FRIENDLY MANAGEMENT OF HADDA BEETLE (*Henosepilachna vigintioctopunctata* F.) (COLEOPTERA: COCCINELLIDAE) USING SLECTED BOTANICAL EXTRACTS

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Introduction

Brinjal (*Solanum melongena* L.) is a major vegetable crop grown in Sri Lanka. Insect pests are the major threat to brinjal cultivation. Hadda beetle (*Henosepilachna vigintioctopunctata* F.) (Coleoptera: Coccinellidae), whis is a polyphagous pest of economically important solanaceae and cucurbitaceae family crops, is a serious insect pest of brinjal cultivation in northern Sri Lanka next to the shoot and fruit borer. The adult and larval stages feed on epidermal tissues of leaves, flowers and fruits of brinjal and cause considerable economic losses [1]. Synthetic insecticides are preferred among the management strategies proposed and frequently being used by the farmers. Recently imposed government policy on organic agriculture to minimize the cumulative adverse effects agrochemicals on human and environment, pushed the farmers to use ecofriendly approaches. Therefore, the primary goal of this research was to study about the insecticidal activity and antifeedant activity of selected botanical extracts against Hadda beetle and qualitative analysis of phytochemicals present in the screened extracts.

Materials and Methods

The investigations were conducted at the Plant Protection & Bio Control Laboratory at the Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, ariviyal Nagar, Kilinochchi (Longitude: 80.4, Latitude: 9.32, Altitude : 46m), belongs to the dry zone. The mean annual rainfall is around 1125mm and the mean annual temperature is around 25-35 ◦C (Department of Meteorology, 2021).

Collection and rearing of Henosepilachna vigintioctopunctata

Adult beetles of *H. vigintioctopunctata* were collected from brinjal farms at Thirunelvely and Kayts of Jaffna district and were reared in the insect rearing cages on the host plants for multiplication.

Preparation of Aqueous extracts

The plant leaves of *Senna alata*, *Calotropis gigantea*, *Cascabela thevetia*, *Vitex negundo* and *Justicia adhatoda* were collected from the herbal garden at the

research veneue and neem (*Azadirachta indica*) seed kernels were bought from an ayurvedic shop. Collected plant parts were surface sterilized with 1% NaOCl and were shade dried for five days under room temperature. Dried plant parts were ground in to fine powder using electric blender under aseptic conditions.
Hot water method of extraction was used for the preparation of plant extracts. Each plant powder of 100 g was dissolved in 1000 mL hot water (60˚C) and mixed well. Then solution was kept at water bath at 85˚C for 2 hrs. The solution was intermittently shaken manually for every 30 minutes. The solution was left to cool and then filtered using muslin a cloth. The solution was centrifuged at 4000 rpm for 10 minutes and the supernatant of the extract was obtained separately and stored in the refrigerator at 4˚C for the experiments.

Testing antifeedant activity
Testing antifeedant activity was performed using leaf disc with no choice method [1]. The experiment was carried out using plastic containers of the same size (3L). Fresh brinjal leaves were collected from Biology home garden and they were cut into 5 cm diameter leaf discs. Leaf discs were dipped into plant extracts and left to air dried. Leaf discs were placed above wetted filter paper to prevent early drying. Then they were placed into plastic containers at the rate of 1 leaf disc/container. Adult beetles of same age and size were collected from the rearing cages. Adult beetles were placed into bottles at the rate of 1/container and the lid was closed. Distilled water treated leaf disc was used as control. Totally seven treatments were provided with ten replicates. Leaf area consumed by the beetle was recorded after 24 hrs and 48 hrs intervals. Graph sheet method was used to find the leaf area consumed. Leaf area consumed in plant treatments were corrected against control. The antifeedant index was calculated using the following formula [2];

$$\text{Antifeedant Index } (\%) = (\text{C-T})\ (\text{C+T})\ \text{X } 100\%$$

C – Leaf area consumed in control
T – Leaf area consumed in treatment

Testing Mortality
Testing mortality was carried out in the same way as testing antifeedant activity. Fresh brinjal leaves were dipped in each extract and placed inside bottles at the rate of one leaf per bottle. Wetted filter paper was placed under the leaf to prevent early drying of leaves [1]. Distilled water was used as control. Ten adult beetles were placed in each bottle and the number of dead beetles were counted at 12 hours, 24 hours and 48 hours of intervals. The mortality rate was calculated by the following formula,

$$\text{Mortality rate} = \text{Number of dead beetles} \text{Number of beetles used for experiment X } 100\%$$

Qualitative analysis of phytochemicals

Secondary metabolites present in the botanical extracts such as alkaloids, phenols, terpenoids, saponin, flavonoids and quinones were measured qualitatively using standard methods [3].

Data collection and Statistical analysis

Complete randomized design (CRD) was used to perform analysis of variance (ANOVA) and Tukey's HSD multiple comparison test was administrated to identify the best treatment at P <0.05 using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The experimental results show that the antifeedant efficacy of different plant extracts were significantly different at P <0.05 (Table 1). Data pertaining to results presented in Table 1 shows that antifeedent efficassy of *C. gigantea*, *C. thevetia* and *J. adhatoda* was 100 % even after 48 hours. *Senna alata* expressed lowest antifeedant index after 48 hours among all other six plant extracts. After 48 hours the antifeedant index of *A. indica* reduced from 100 % to 78.58 %.

Table 1: Antifeedant index after 24 hours and 48 hours

Plant extract	After 24 hours	After 48 hours
Azadirachta indica	100 %	78.58 %
Senna alata	85.24 %	56.5 %
Calotropis gigantea	100 %	100 %
Cascabela thevetia	100 %	100 %
Vitex negundo	80.71 %	74.23 %
Justicia adhatoda	100 %	100 %
Water	15.75%	5.8%

Values are means of four replicates based on the formula of calculating antifeedant index

Insecticidal activity of crude extracts was significantly on par compared to the control treatment at P <0.05. The mortality percentage of *H. viginitioctopunctata* after 12 hours, 24 hours and 48 hours were varied from 2.5±5-12.5±12.58, 2.5±5-30±14.14 and 2.5±5-62.5±15, respectively (Table 2). The highest mortality rate was observed for *A. indica* at each time intervals. High mortality rate of insects normally indicates the higher insecticidal activity.

Table 2: Mean mortality percentage of adult beetles against different plant extracts

Plant extract	After 12 hours	After 24 hors	After 48 hours
Azadirachta indica	12.5 ± 12.58^{a}	30 ± 14.14^{b}	62.5 ± 15^{b}
Senna alata	7.5 ± 5^{a}	12.5 ± 5^{ab}	30 ± 8.16^{b}
Calotropis gigantea	5 ± 5.77^{a}	27.5 ± 12.58^{abc}	50 ± 14.14^{c}
Cascabela thevetia	10 ± 8.16^{a}	15 ± 12.91^{abc}	25 ± 10^{c}
Vitex negundo	2.5 ± 5^{a}	5 ± 5.77^{bc}	15 ± 5.77^{c}
Justicia adhatoda	5 ± 5.774^{a}	12.5 ± 9.57^{c}	27.5 ± 9.57^{bc}
Control (Distilled water)	2.5 ± 5^{a}	2.5 ± 5^{c}	2.5 ± 5^{bc}

Values are means of four replicates. Within the column similar alphabets are statistically not significant according to the Tukey's HSD multiple comparison test at $\alpha = 0.05$.

As per the qualitiative analysis of the phytochemicals present in the different botanical extracts, Tannins and Saponin are present in all the extracts. All the phytochemicals tested were present in *A. indica* and *C. gigantea* except the Coumarin which is not present in *A. indica* (Table 3). Phenols were absent in *S. alata* and *V. negundo*.

Table 3: Screening for phytochemicals present in plant extracts

Phytochemicals	***Azadirachta indica***	***Senna alata***	***Calotropis gigantea***	***Cascabela thevetia***	***Vitex negundo***	***Justicia adhatoda***
Phenols						
Iodine test	+	-	+	+	-	+
Potassium dichromate test	+	-	+	+	-	+
Alkaloids	+	-	+	+	+	+
Tannins	+	+	+	+	+	+
Saponin	+	+	+	+	+	+
Coumarin	-	-	+	+	-	+
Flavonoids						
Conc H_2SO_4	+	+	+	-	-	-
Ammonia test	+	+	+	-	-	-

+ = present - = absent

Saljoqi *et al.* [3] reported that botanical extracts of *Melia azadarach*, *Perthenium hysterophorus*, *Phlogocanthus thyrsiflorus*, *Vitex trifolia*, *Zanthoxylum acanthopodium*, and *A. indica* were tested on rice weevils and M. azadarach caused highest mean mortality of 80.54% at 35 days after treatment. Aqueous leaf extracts of *Calotropis gigantea* outperformed *Croton laccifera* in terms of mortality and reduction of fecundity of 1st and 2nd instar nymphs, and newly emerged apterous females of cowpea aphid (*Aphis craccivora*) [4]. Among the various herbs, *A. indica* based insecticides has been the most accepted bio-pesticides and It acts as an antifeedant, repellent, and repugnant agent and induces sterility in insects by preventing oviposition and interrupting sperm production in males [5].

Conclusions and Recommendations

Botanical extract *C. gigantean* and *A. indica* are best botanicals among the tested showing highest antifeedant and mortality against *H. viginitioctopunctata*. various phytochemicals present in them are responsible for the insecticidal properties. Quantitative anlysis and repetitive field studies are recommended for recommendation.

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SENSORIAL QUALITIES OF THREE COMMON FOOD COMMODITIES COOKED WITH DIFFERENT TECHNIQUES

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Introduction

Cooking involves the application of heat to prepare food for consumption. Over the years, people have discovered several energy sources suitable for cooking purposes. Widely used energy sources include biomass (such as firewood), petroleum-based products (such as kerosene and liquefied petroleum gas (LPG)), and electricity. There are some discussions around these cooking techniques related to their effect on the sensorial properties and nutritional properties of cooked food and there is a significant void of scientific information on the credibility of such claims. This study aims to explore the effect of the cooking energy sources on the key sensorial properties of the final cooked products.

Materials and Methods

Cooking experiments were carried out using a firewood stove, kerosene burner, LPG burner (Model-IGCSB 001, Innovex, Sri Lanka), hot plate (Model-AO9602, 1500W, Astro, China), Induction cooker (Model-IND-4310, 2000W, Prince, India) and grill microwave oven (Model-NNGT342M, 800W, Panasonic, Japan). Rice, fresh carrots and potatoes were purchased from the same local stores in Colombo district.

Sample preparation

For the experiments involving rice, 150g of rice was measured and rinsed with tap water, then cooked with 600mL of excess water using each method. As mentioned by Lakshmi et al. [1], the end point of cooking was identified parallel glass method. Here, periodically drawn rice samples were pressed in between two small glass plates. When there was no white core observed, the sample was considered to be completely cooked. Then excess cooking water was drained off as much as possible. The rice was simmered until completely cooked.

For the experiments involving potatoes and carrots, raw potatoes and carrots were peeled and washed and cut into 1cm×1cm cubes. For potatoes, a 100g sample was measured and cooked in 300 mL of boiling water. Similarly, 100g aliquots of carrot pieces were measured and cooked in 200 mL of boiling water using each cooking method. The level of cooking was determined by the benchmarks set during the preliminary trials which were defined based on the texture, according to local eating habits. For all the cooking experiments, the pots were covered with a lid.

The cooking temperature was maintained between 100 °C and 95 °C by halting the heat input at boiling and continuing when the temperature dropped beneath 95 °C [2]. The experiments were carried out in five replicates.

Sensory evaluation

All samples were subjected to sensory analysis in terms of colour, odour, taste, aftertaste, mouthfeel and overall acceptability. The sensory analysis was performed by 30 semi-trained panelists. The intensity of the properties was determined using a 9-point hedonic scale where 1 extremely dislikes and 9 is extremely likes [3].

Statistical analysis

The obtained data were analyzed using IBM SPSS Statistics 21 software. In each case, mean values were calculated. A multiple comparison was performed for the data obtained using Tukey's HSD test. For all statistical analyses, differences were considered significant at $p < 0.05$.

Results and Discussion

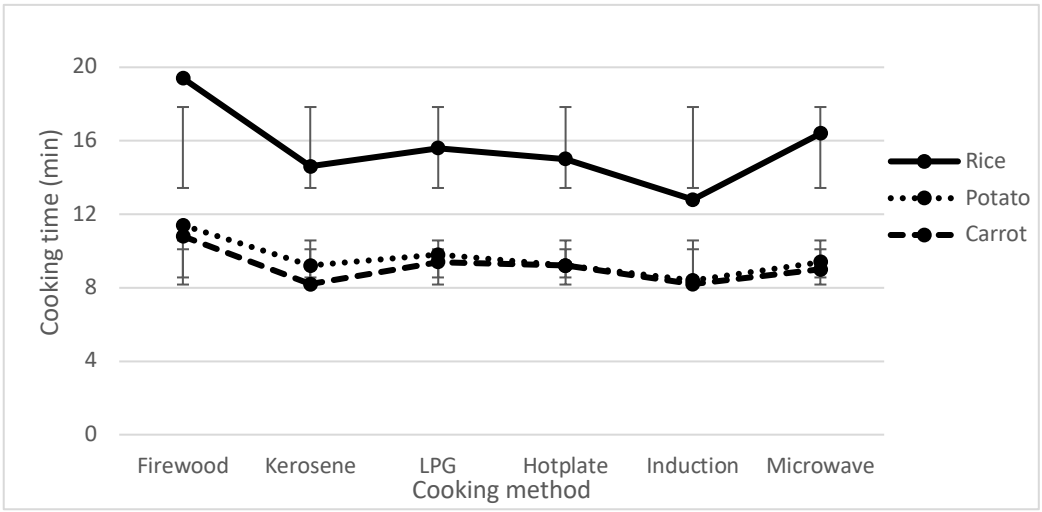

Figure 1. Mean cooking times of rice, potato and carrot cooked with each method

Figure 1 illustrates the mean values of the cooking times of each method. According to the statistical analysis, firewood cooking shows a significantly higher time duration for each component (19.40±0.55 min for rice, 11.40±0.55 min for potato, 10.80±0.84 min for carrot), due to the low combustion efficiency. In rice, induction cooking resulted in significantly low cooking time (12.80±0.84 min) compared to the other methods.

Table 1 shows the mean score values obtained from the sensory analysis of each food sample.

Table 1. Mean score values obtained from the sensory analysis for rice, potato and carrot

Food item	Cooking method	Mean score values					
		Colour	Odour	Taste	Aftertaste	Mouthfeel	Overall
Rice	Firewood cooking	5.3^{a}	4.2^{a}	5.0^{a}	4.2^{a}	4.6^{a}	4.7^{a}
	Kerosene cooking	7.8^{bc}	7.4^{b}	7.3^{bc}	7.4^{b}	7.4^{b}	7.6^{b}
	LPG cooking	6.2^{abc}	6.3^{b}	6.3^{ab}	6.3^{ab}	6.1^{ab}	6.3^{ab}
	Hot plate	5.1^{a}	6.1^{b}	6.0^{ac}	6.1^{ab}	6.1^{ab}	6.0^{ab}
	Induction cooking	7.5^{c}	7.1^{b}	7.2^{bc}	7.1^{b}	6.9^{ab}	7.4^{b}
	Microwave	5.3^{a}	6.7^{b}	6.4^{ab}	6.7^{b}	6.3^{ab}	6.0^{ab}
Potato	Firewood cooking	7.4^{a}	6.3^{a}	6.5^{a}	6.2^{a}	6.6^{a}	6.2^{a}
	Kerosene cooking	6.7^{a}	6.0^{a}	6.8^{a}	6.2^{a}	6.4^{a}	6.4^{a}
	LPG cooking	7.3^{a}	6.1^{a}	6.9^{a}	6.5^{a}	6.3^{a}	6.7^{a}
	Hot plate	6.1^{a}	6.2^{a}	6.5^{a}	6.5^{a}	6.8^{a}	6.8^{a}
	Induction cooking	7.6^{a}	7.1^{a}	7.2^{a}	7.1^{a}	7.2^{a}	7.3^{a}
	Microwave	6.2^{a}	6.7^{a}	6.5^{a}	6.4^{a}	6.2^{a}	5.9^{a}
Carrot	Firewood cooking	7.9^{a}	6.4^{a}	5.1^{a}	4.6^{a}	4.6^{a}	5.9^{acf}
	Kerosene cooking	8.1^{a}	6.5^{a}	7.5^{bc}	6.8^{ab}	7.2^{b}	7.7^{b}
	LPG cooking	6.9^{ab}	7.4^{a}	7.6^{c}	6.9^{ab}	7.6^{b}	7.2^{bf}
	Hot plate	5.6^{b}	6.1^{a}	5.5^{ab}	5.9^{ab}	5.9^{ab}	5.2^{ad}
	Induction cooking	6.2^{b}	5.7^{a}	6.8^{abc}	6.3^{ab}	6.5^{ab}	5.7^{acf}
	Microwave	8.0^{a}	6.9^{a}	7.0^{abc}	7.1^{b}	6.3^{ab}	7.5^{be}

$^{a-f}$ Values represent mean scores with the same superscript letters within the same column that are not significantly different for each commodity (P>0.05).

The mean score values of the sensory analysis revealed that the kerosene cooked sample was most preferred concerning colour (7.8), odour (7.4), taste (7.3), aftertaste (7.4), mouthfeel (7.4) and overall acceptability (7.6) in rice. Firewood stove-cooked rice obtained the lowest mean score values for odour (4.1), taste (5.0), aftertaste (4.2), mouthfeel (4.6) and overall acceptability (4.7) indicating

that it was the least preferred. In addition to that, a significant difference ($p<0.05$) was recorded in the mean score for odour (4.1).

This apparent difference is attributed to the characteristic woody/ smoky smell in the firewood-cooked food. Especially, rice being more sensitive to odours, it intensively absorbs the smell of the firewood smoke, masking the actual smell as well as the taste of rice and making it less desirable. However, the kerosene smell does not seem to affect rice samples unacceptably, probably due to lower exposure time resulting in a short cooking duration compared to firewood and a fairly efficient stove design.

No significant differences ($p>0.05$) were observed in mean score values, indicating similar sensory qualities in firewood, kerosene, LPG, hotplate, induction and microwave-cooked potato samples. Nevertheless, induction cooking was revealed to be most preferred for potatoes as it obtained higher mean score values (7.1-7.6) for all attributes. Similar results were obtained by Martínez-Gómez et al. [4] for oranges with oatmeal, grilled chicken and broccoli cooked in induction and LPG cookers. As a result of the low duration of heat treatment in induction cooking, less thermal damages happen to the cell structure of the food and less volatile evaporation occurs, which may benefit the sensory qualities.

In carrots, higher mean score values were given for LPG cooking in terms of odour (7.4), taste (7.6) and mouthfeel (7.6), while, kerosene cooking obtained higher score values for colour (8.1) and overall acceptability (7.7). In terms of aftertaste, the microwave-cooked sample obtained the highest mean score (7.1).

Conclusions and Recommendations

The study has shown that the cooking time varies depending on the cooking technique used. Firewood cooking is accountable for longer cooking durations for all rice, potato and carrot. In terms of sensory qualities, kerosene cooking is preferred for rice and carrot. Meanwhile, induction cooking was found to be more acceptable for potatoes in terms of sensory qualities. However, to identify the acceptability of the energy source on the overall quality of the cooked foods, further evaluations on nutritional properties of the cooked food as well the heat transfer characteristics of the stove should be conducted which will unravel more interesting information. A cost analysis also needs to be done to calculate the effectiveness of each technique.

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A STUDY ON CORRELATION BETWEEN FOOD HABITS AND IRON DEFICIENCY ANAEMIA AMONG CHILDREN AT BASE HOSPITAL KALMUNAI NORTH

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Introduction

The utmost communal nutritional problem in the world is iron deficiency anemia. The incidence varies across the globe, with greater rates in developing nations [1]. Anemia affects above 30% of the global population, primarily owing to a lack of iron [2]. With the exception of China, where the frequency of IDA is lower, IDA is also an issue across Latin America, Middle East, Caribbean, East Asia, and Pacific, where the prevalence of IDA ranges from 22 to 66 %[3]. Anemia continues to be a major global health issue, impacting 43% of children under age of five, 38% of pregnant women, and 29% of non-pregnant women globally [4]. Southern Asian and African children are especially vulnerable, with IDA affecting more than 50% children of preschool age in most nations.

Anaemia develops when the body's physiologic requirements for red blood cells (and as a result, their carrying capacity for oxygen) are not met. Globally, ID is thought to be the most significant reason of anemia. [5]. A lack of iron Anemia is a condition in which there isn't enough iron in the body to maintain normal red cell production[6].Although the frequency of iron deficiency anemia is about equal in girls and boys, mother's anemia during pregnancy, and a lack of breast - feeding all contribute to an increased occurrence of the disease in children [7].

Iron deficiency mainly affect newborns and young children in most parts of the world, due to their greater iron requirements associated to growth [8]. Young kids are particularly susceptible to the effects of IDA since their body systems are working to develop, particularly their brains, which are the quickest developing organs during infancy and early childhood. [2]. It can cause major public health problems, such as increased disease and mortality in children, as well as impaired growth, immune system, and cognitive development, decreased physical activity, low endurance capacity, and poor learning ability[2], [9], [10].

In Sri Lanka, anaemia has continued to be an important public health problem as shown in many studies. Even though there are few studies in the field of nutritional deficiency Anaemia in Sri Lanka, there are no studies done in Ampara district to portray the incidence of IDA among children. Hence this study was conducted to evaluate the correlation between food habbit and IDA among children aged 1-14years at Base hospital, Kalmunai North.

Materials and Methods

This was a cross sectional analytical study carried out at the paediatric clinic and paediactric ward of Base Hospital, Kalmunai North over a period of 6 months from January to june2022. The ethical clearance was taken from the Ethics review committee, FHCS, EUSL. Written informed consent was obtained from children and parent before enrolling them into the study. This study was conducted on 101 children in the age group of 1-14 years who attended the Paediatric clinic and admitted to Paediatric ward at Base Hospital Kalmunai North, during research period. The children previously transfused with blood within 120 days and currently consuming multivitamin and/or mineral supplements on a regular basis were excluded from the study. The interviewer administered questionnaire was validated with food frequency and dietary data were collected. After the diagnosis, study patients were subjected to the full blood count, serum ferritin (SF) level and C - reactive protein (CRP). WHO defined cut-off levels were used to assess the anaemia (Hb < 11g/dl) iron deficiency (SF < 15 µg/l).

Statistical analysis

Appropriate data entry and statistical analysis were performed on Microsoft excel using social package for social science (SPSS) version 21.0. Data was summarized using descriptive statistics and food habbit was evaluated by pearson Correlation. Proportions were considered statistically significant at 95%. The associations was made by using appropriate statistical test (p-value). Statistical significance were set at $p < 0.05$.

Results and Discussion

Fruits

Table 01. Results of Pearson correlation analysis - fruits

Fruits	Significant (2 tailed)	Pearson correlation
Orange/Sour orange	.032	-.214*
Banana	.329	.098
Papaya	.090	-.170
Guava	.006	-.272**
Dates	.023	-.226*
Star gooseberry	.328	-.098
Pomegranate	.050	-.195

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

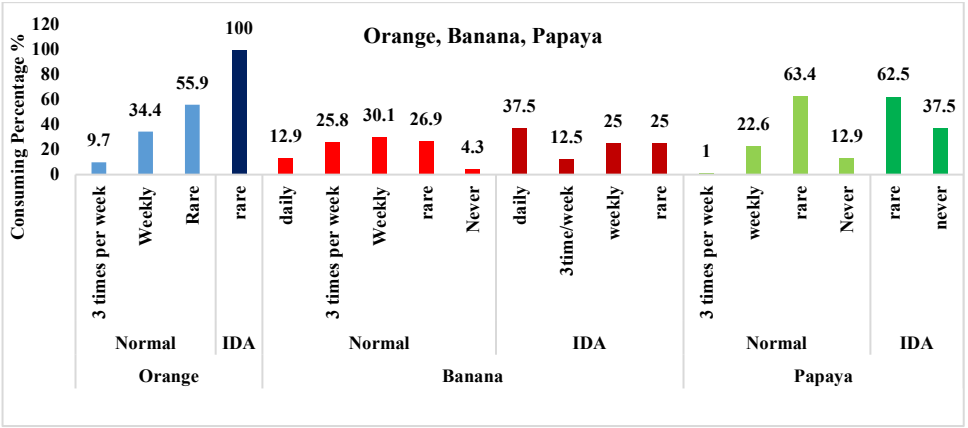

Figure 1. Quantity of consumption of orange, banana and papaya

The consumption of oranges by the IDA and normal groups varied considerably ($p < 0.05$), as shown in figure1. Children who had regular consumption oranges were less likely to have IDA. It might be due to the high content of vitamin C of oranges, which increases iron absorption [11]. Papaya and banana consumption between the IDA and normal groups, did not show any significant difference in both groups.

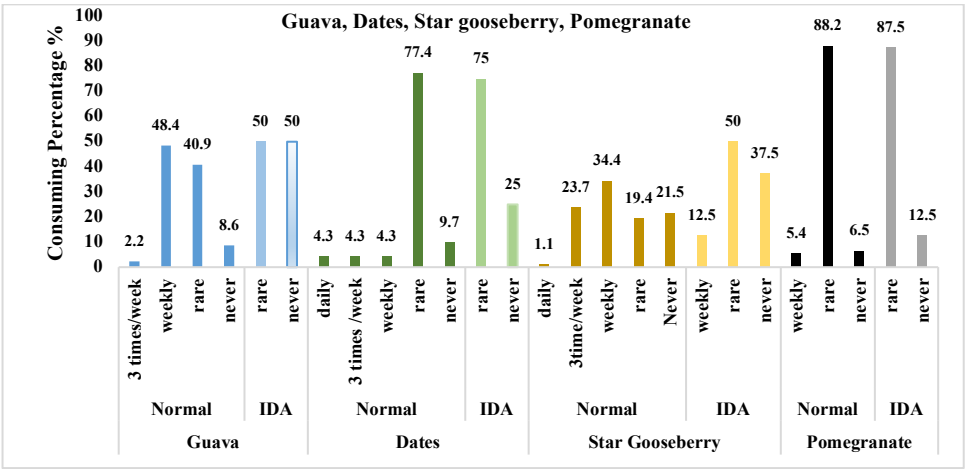

Figure 2. Consumption of guava, Dates, star gooseberry and pomegranate

Guava was significantly varied ($p < 0.01$) in the normal and IDA group, as shown by figure 2. Children who are having guava seldom were more susceptible to IDA. Dates between the normal and IDA groups were significant ($p< 0.05$). Because dates are high in iron, the kids who regularly ate them were not impacted by IDA.

Star gooseberry and pomegranate did not differ significantly between the two groups.

Vegetables

Table 02. Results of Pearson correlation analysis - Vegetables

Vegetables	Significant (2 tailed)	Pearson correlation
Green leafy vegetable	.004	-.282**
Beans	.647	-.046
Tomato	.323	-.099
Potato	.505	.067
Beets	.511	.066
Leeks	.137	-.149

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

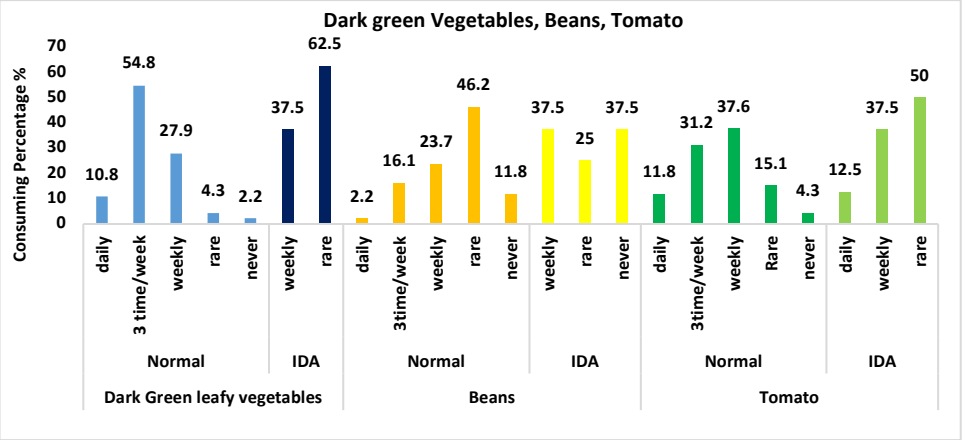

Figure 3. Consumption of dark green leafy vegetables, beans and tomato.

Dark green leafy foods considerably varied between the normal and IDA groups, as shown in figure 3. Because they are rich in iron, children who consumed dark green leafy vegetables more frequently were less likely to get IDA. Tomato and beans did not significantly differ between the two groups.

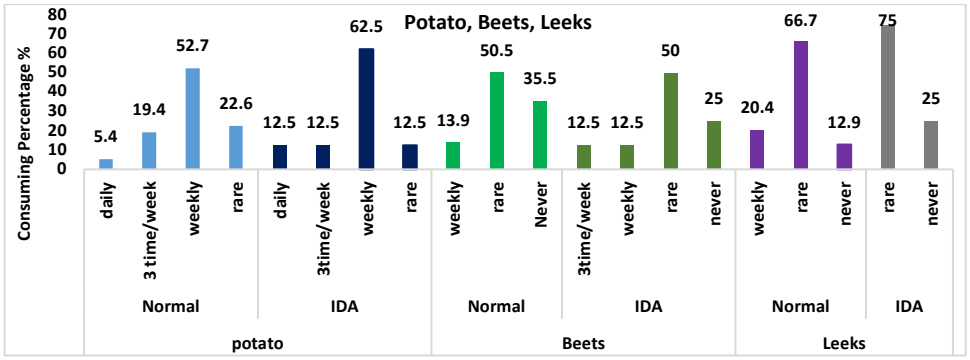

Figure 4. Consumption of potato, beets and leeks

According to the figure 4, potato, beets and leeks were not significantly differed among normal and IDA groups.

Animal products

Table 03. Results of Pearson correlation analysis - Animal products

Animal products	Significant (2 tailed)	Pearson Correlation
Fish	. 000	-.379**
Chicken	.000	-.463**
Liver	.000	-.371**
Meat	.000	-.395**
Shrimp	.005	-.280**
Egg	.043	-.202*

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

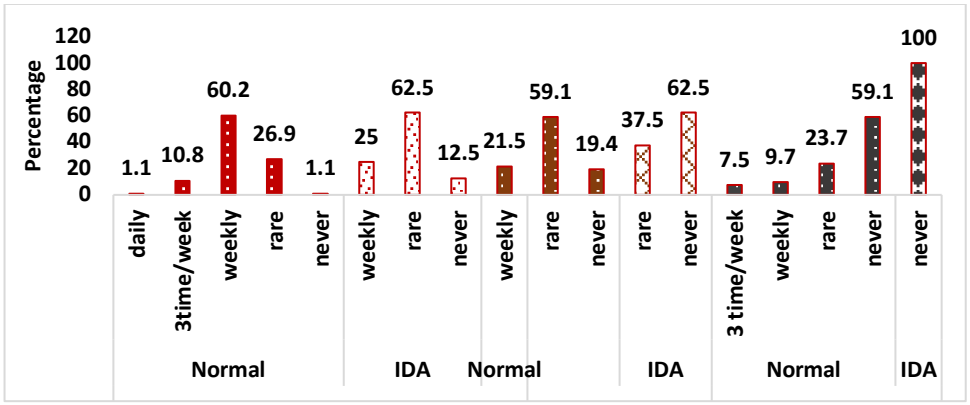

Figure 5. Quantity of Consumption of chicken, liver and meat

Chicken, liver, and meat were significantly different between the normal and IDA groups, as shown in graph 5. The children who consumed chicken, liver and meat very rarely were affected by IDA than others. Considering that these foods are abundant in heme iron [12].

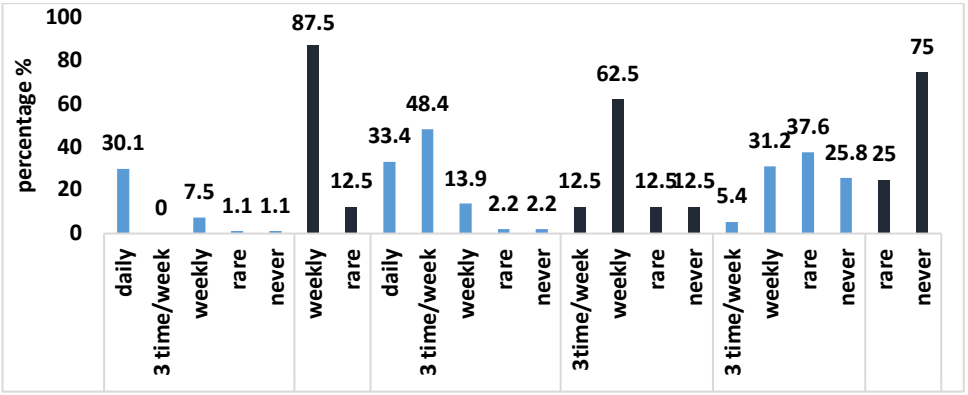

Figure 6. Quantity of consumption of fish, egg and shrimp

Fish, eggs, and shrimp were significantly different between the normal and IDA groups, as shown in figure 6. More eggs were taken than fish or shrimp. Children who consumed more fish, eggs, and shrimp were not impacted by IDA since these foods contain heme iron [12].

Dairy products

Table 04. Results of Pearson correlation analysis – Dairy products

Dairy products	Significant (2 tailed)	Pearson correlation
Yoghurt	.117	.157
Chocolate	.006	.274**
Cheese	.021	.229*

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

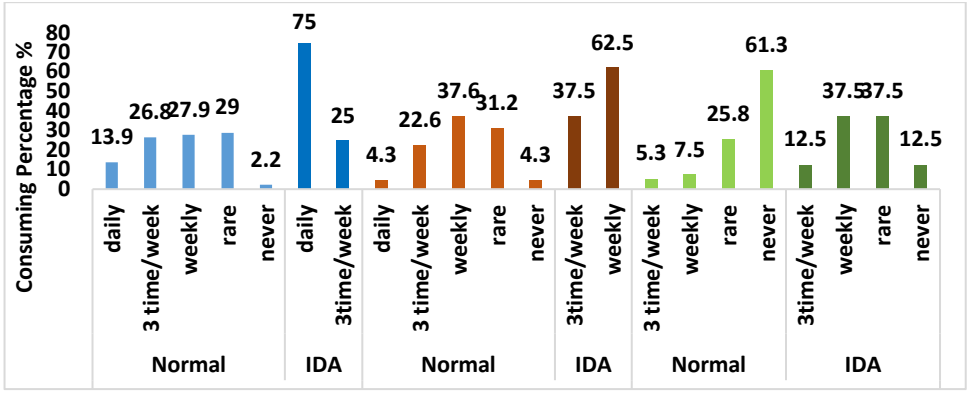

Figure 7. Consumption of chocolate, yoghurt and cheese

Chocolate and cheese greatly varied between the normal and IDA groups, as shown in figure 7. IDA was more prevalent in children who had cheese and chocolate more regularly. The polyphenol in chocolate and the calcium in cheese could be to reason. Both reduce the absorption of iron [13], [14].

Drink

Table 05. Results of Pearson correlation analysis - Drink

Drink	Significant (2 tailed)	Pearson correlation
Black tea	.004	.283**
Fresh milk	.001	.326**
Coffee	.193	.131

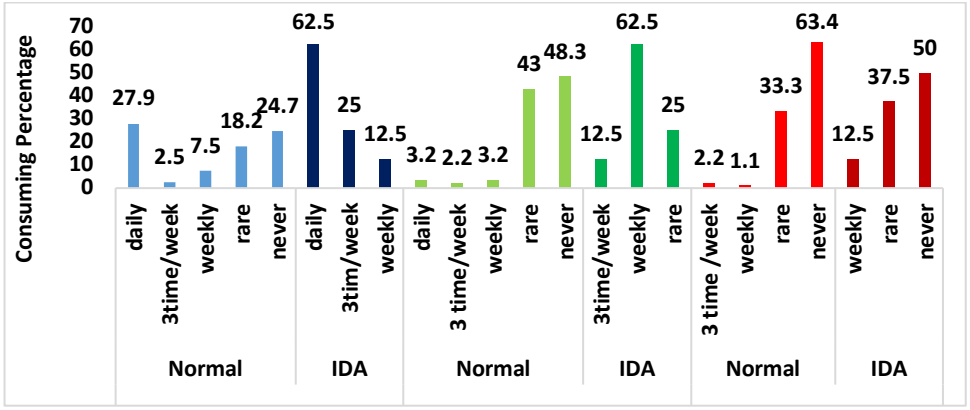

Figure 8. Consumption of black tea, fresh milk and coffee

According to figure 8, black tea and fresh milk were significantly different among normal and IDA group. The children who consumed more black tea and fresh milk

had IDA than normal people. It may because of high level of calcium in those food which reduce the absorption of iron [14].

Conclusions

The food consumption including orange, Guava, Dates, Dark green leafy vegetables, chicken, liver, fish, meat, egg, shrimp, chocolate, cheese, black tea and fresh milk were significantly affected the Hb level. IDA could be prevented or reduced by changing the food habits among the children.

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DEVELOPMENT OF AN EDIBLE COMPOSITE COATING USING PLANT EXTRACTS TO PROLONG THE POSTHARVEST LIFE OF TOMATOES

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Introduction

Food industries have driven various approaches to reduce postharvest losses of fruits and vegetables by applying diverse technologies. Since many postharvest changes within the crops cannot be avoided, remedies such as natural coatings are practiced to maintain the quality of fruits and vegetables by reducing the rate of biological processes such as respiration, transpiration, and ethylene production linked to ripening and senescence. Tomato (*solanum lycopersicum L.*) of Solanaceae family is a climacteric fruit with a comparatively shorter postharvest life; however, consumed with many meals around the world due to great deal of composition rich with vitamins and beta-carotene. Although the tomatoes are often harvested at the mature green stage, fruits ripen spontaneously throughout the supply chain causing excessive postharvest loss along with the gradual deterioration of final fruit quality. In tropical countries, temperature and humidity are the main factors correlated with the postharvest deterioration of tomatoes. Since cold storage is a bit expensive to practice in countries like Sri Lanka, the edible coating is an affordable substitute to prolong the postharvest life of fresh commodities, controlling the biochemical changes at different maturity stages. The edible coating applied on the tomato epidermis creates a modified atmosphere around the fruits and thereby controls moisture loss, gas transmission, fluctuations of biochemical properties and other detrimental effects on quality loss during storage.

Literature reported the incorporation of variety of plant extracts with many health benefits in formulating the coating blends for application of different fruits and vegetables. Dawulkurundu (*Neolitsea cassia*) is one of the underutilized plant species available in Sri Lanka, which gives gum-like viscous leaf extract containing polysaccharides that can be easily utilized in coating preparation. According to the National Aquatic Resources Agency (NARA) of Sri Lanka, there are different varieties of seaweeds found along the coastline areas, however, only very few are utilized for economic purposes. Alginate is the principle biomacromolecule in the

cell wall of brown seaweed and it has a linear molecular structure that can form a strong polymer matrix and structures important in film forming [1]. In case of *Aloe vera*, among all health benefits, antibacterial and antioxidant properties are promisingly a value addition to a food coating

Hence, this study was conducted to develop natural edible coatings for fresh tomatoes using selected underutilized plant leaves available in Sri Lanka to substitute the expensive preservation techniques. Further, effectiveness of the formulated natural coatings on postharvest life of tomato during storage under ambient conditions was evaluated.

Materials and Methods

Preparation of the plants extracts

The main plant ingredients, *Neolitsea cassia* (Dawulkurundu) and *Aloe vera* leaves were collected from available areas of Kurunegala district, while brown seaweed, *Sargassum crassifolium,* was collected from the coastal area of Hikkaduwa. *Neolitsea cassia* leaves were cut into small pieces, blended and squeezed through a cotton cloth. *Aloe vera* gel was extracted after removing the spikes from leaves and separating the inner leaf gel. The separated gel was crushed in a blender and filtered to remove the fibrous fraction [2]. Alginate extraction from *Sargassum crassifolium* was followed using the hot extraction method of Chee et al [3] with slight modifications.

Preparation of the edible coating blends

Plants extracts (Dawulkurundu extract, *Aloe vera* gel and Alginate) were mixed with the plasticizer glycerol in eight different combinations following the Taguchi Orthogonal Array Design ratios (Table 1). These blends were heated to 50 °C and mixed well using an orbital shaker (Model VRN-480, Taiwan) for 1 hour until all ingredients were well dissolved to prepare homogeneous coating blends. Then the formulated coatings were stored under refrigerated conditions (4 °C) until further use.

Table 1. Ratio of coating formulations based on Taguchi Orthogonal Array Design

	Alginate	Dawulkurundu	*Aloe vera*	Glycerol
Coating 01 (C1)	1	1	1	1
Coating 02 (C2)	1	2	2	2
Coating 03 (C3)	1	2	1	2
Coating 04 (C4)	1	2	2	1
Coating 05 (C5)	2	1	1	2
Coating 06 (C6)	2	1	2	1
Coating 07 (C7)	2	2	1	1
Coating 08 (C8)	2	2	2	2

Determination of the moisture content and water solubility of coatings

The moisture content of the coatings was determined using the standard oven drying method. The coatings were dried at 105 ◦C for 24 hours until the dried films reach equilibrium weight. Water solubility of the coatings was measured according to the gravimetrical method [4], where dried coating samples were cut into equal pieces and dried at 75 ◦C until constant weight. Then the samples were immersed in distilled water for 24 hours. The undissolved film was filtered and recovered at 105 ◦C for 24 hours to find the most water-resistant coatings.

Application of coatings on tomatoes

Disease free tomatoes (*Solanum lycopersicum* L.) of same variety in similar green maturity stage and equal size (diameter around 3.5 cm), were selected for the coating process. First, tomatoes were gently washed with tap water and air dried to detach any impurities. Coating application was done using the dipping method, where the tomatoes were directly immersed in the coating solution for 2 minutes followed by air drying and then stored under room temperature (27 ◦C) for eighteen days period. Control of uncoated tomato samples too were stored under same conditions. All treatments were conducted in five replicates.

Determination of the postharvest weight loss of tomatoes

Postharvest weight loss of tomatoes under each coating was determined by monitoring the weight loss of coated tomatoes in 3 days intervals for eighteen days of storage period, until the tomatoes were unfit for the human consumption. The percentage weight loss was calculated as the difference between the initial weight and final weight of the fruit and expressed based on the initial weight.

Assessment of the chemical properties

Based on the above results of pretesting, tomatoes coated with the best three coatings (C6, C7 and C8) were tested for titratable acidity (TA), total soluble solids (TSS) and pH in three days intervals for eighteen days along with the control treatment. Tomatoes were crushed in a blender and filtered to obtain a homogeneous sample for analysis. The TSS were measured using a digital brix meter (Model 38-A1, UK) and pH was measured using digital pH meter. The titratable acidity was measured by titrating 10 ml of tomato extract with 0.1N NaOH using phenolphthalein as the indicator.

Statistical analysis

All data were statistically assessed using IBM SPSS Statistics 21 software and the significant difference among the treatments were analyzed by One-Way ANOVA mean comparison, done for each coating with the control at a confidence level of $P < 0.05$.

Results and Discussion

Moisture content and water solubility of coatings

According to Table 2, the moisture contents of the coatings ranged from 71.01±2.1% to 86.69±1.83%, and there was a significant difference ($P<0.05$) between the C3 and C4, the lowest and the highest moisture contents, respectively. Moisture content of the coatings was directly proportional to the *Aloe vera* content in the coating blends since the gel includes more water compared to the other plant extracts used. The lowest water solubility reported was 81.80±1.14%, from coating 7 with a high proportion of alginate and dawulkurundu in the blend (33.33% each). Although there was no significant difference ($P>0.05$) among the water solubility of the eight coatings, the incorporation of more alginate (≥ 25%) and dawulkurundu (≥ 25%) reduced the water solubility and improved overall coating properties. Alginate is a film-based material with a good film-forming capacity. The physical properties of alginate are decided by the presence of mannuronic acid and guluronic acid units, which effects the gel strength and elasticity [5].

Table 2. Moisture content, water solubility of the coatings and postharvest weight loss of coated tomatoes.

Coating Number	Moisture Content %	Water solubility %	Postharvest weight loss %
Coating 01	85.64±1.84^{ab}	86.39±1.8^{c}	28.75±1.67^{d}
Coating 02	75.07±1.19^{ab}	92.61±1.27^{c}	27.76±1.88^{d}
Coating 03	71.01±2.1^{a}	93.78±1.51^{c}	24.95±1.04^{e}
Coating 04	86.69±1.83^{b}	87.53±1.34^{c}	25.46±1.19^{e}
Coating 05	73.71±1.92^{ab}	87.29±1.04^{c}	24.81±0.53^{e}
Coating 06	85.11±1.31^{ab}	86.43±2.72^{c}	23.06±0.59^{e}
Coating 07	86.61±1.74^{ab}	81.80±1.14^{c}	23.68±1.77^{e}
Coating 08	83.53±1.33^{ab}	86.63±1.21^{c}	21.58±0.31^{e}
Control	-	-	35.95±0.52^{d}

$^{a-e}$ Values represent mean scores with the same superscript letters within the same column are not significantly different (P>0.05).

Postharvest weight loss of tomatoes

The weight loss increased in all coated and non-coated tomato samples during storage as expected (Table 2). There was a significant difference between the postharvest weight loss of coated tomatoes compared to the control. The lowest postharvest weight loss (21.58±1.02%) was recorded for the coating 8, owing to good barrier properties of coating made from the combination of *Aloe vera*. Dawulkurundu, Alginate and Glycerol (2:2:2:2). Transpiration and respiration are two main reasons for high moisture loss in fruits and vegetables resulting in high postharvest mass loss. When tomatoes are treated with a thin layer of the edible coating, it acts as a semi-permeable barrier between the treated samples and the

surrounding environment, reducing the exposure to stress conditions and delaying the migration of moisture, solutes and gaseous exchange to the external atmosphere.

Table 3. Changes in chemical properties of tomatoes

	Day 1	Day 18
pH		
C	3.91±0.02[a]	4.70±0.01[b]
C6	3.91±0.02[a]	4.57±0.02[c]
C7	3.90±0.01[a]	4.55±0.01[c]
C8	3.89±0.01[a]	4.50±0.01[c]
Total Soluble Solids		
C	4.27±0.06[d]	5.80±0.00[e]
C6	4.20±0.10[d]	5.27±0.06[f]
C7	4.23±0.06[d]	5.03±0.05[g]
C8	4.26±0.06[d]	5.00±0.10[g]
Titratable Acidity		
C	0.612±0.00[h]	0.439±0.00[i]
C6	0.614±0.00[h]	0.483±0.00[j]
C7	0.613±0.00[h]	0.491±0.00[k]
C8	0.613±0.00[h]	0.495±0.00[k]

[a-k] *Values represent mean scores with the same superscript letters within the same column are not significantly different (P>0.05*).

pH

pH of both coated tomatoes (C6, C7, C8) and the control were almost equal in day 1 and found to increase during the storage period (Table 3). That is due to the breakdown of acids with respiration during storage. Coated fruits reported only small variations in pH than the control. The coatings reduce the respiration rate and thereby control the breakdown of acids and minimize pH fluctuations.

Total Soluble Solids

Two treatments (C7 and C8) showed a significantly lesser increase of total soluble solids of tomato while the control sample reported a rapid increase of TSS during storage (Table 3). By controlling respiration, coatings slow the use and synthesis of metabolites and also control ethylene production, suppressing the TSS changes.

Titratable Acidity

This is a measurement of the content of citric acid present in tomatoes. The titratable acidity of both coated and non-coated tomatoes decreased with storage period (Table 3). A significant loss of acidity was reported in the control

sample while the treated samples showed only a gradual decrease. Organic acids like citric acids are predominant in respiration. Higher respiration rates in control tomato samples during storage increase the utilization of organic acids, eventually decreasing the titratable acidity. Coating decrease the respiration rate which consequently controls the consumption of acids during storage.

Conclusions and Recommendations

Overall, the most effective coatings were formulated with a high proportion of Alginate and Dawulkurundu, which created semi-permeable membranes sustaining the quality attributes of tomatoes along with a significant impact on reducing the postharvest quantity loss. All coated samples showed minimal fluctuations of pH, TSS, TA and weight loss and the best coating combination is Dawulkurundu: Alginate: Aloe vera: Glycerol, 2:2:2:2 (C8).

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POTENTIAL OF *Nostoc* sp. AS A BIOFERTILIZER ON GROWTH AND YIELD OF PADDY - *Oryza sativa*

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Introduction

Fertilizers play a crucial role to enhance the crop growth as well as the crop production. Until the recent past, most of the farmers have been solely dependent on inorganic fertilizers. Over application of inorganic fertilizers leads to several problems such as different environmental and health issues [1]. The changing global and local socio-political conditions demand shift fertilizer usage from sole inorganic to other alternative ways.

Biofertilizers are the ecofriendly novel tools in agriculture. Microalgae act as nutrient-rich bio resources. Cyanobacteria, also known as blue-green algae, are one of the most popular prokaryotic groups that can photosynthesize and fix atmospheric nitrogen. Incorporating nitrogen fixing cyanobacteria which are rich in macro and micro nutrients to the paddy fields would enhance the productivity, thus it would provide sustainable and ecofriendly solutions to the prevailing fertilizer related issues in paddy cultivation. However, cultivation of cyanobacteria also requires medium which demands chemicals. There has been research on cultivation of different cyanobacteria in a range of waste water [2].

In this background, this current study was conducted with the overall objective of assessing the potential of cultivating *Nostoc* sp. in kitchen wastewater and use the freshbiomass in combination with either Department of Agriculture (DOA) recommended inorganic or organic fertilizer on the growth and yield of paddy.

Materials and Methods

Nostoc sp. already isolated from local environments was obtained from the Microbiology and soil ecosystems research laboratory, at the National Institute of Fundamental Studies, Hantana Road, Kandy.

Semi mass culturing

Nostoc sp. was cultured in 10 L aspirator bottles to obtain sufficient amount of wet biomass in selective diluted kitchen wastewater up to four weeks. Waste water (5 L) consisting of rice wash water and dhal wash water and tap water at the ratio of 4:1:15 was used for cultivation. This ratio was selected from a preliminary study with different ratios of waste water.

Pot experiment

A pot experiment was conducted to assess the potential of wastewater grown *Nostoc* sp. as biofertilizer for paddy cultivation. The treatments are detailed in Table 1. The experimental design was CRD with three replicates. Each pot was

filled with two kg of soil, sampled from a paddy field, air dried and passed through a 2 mm sieve. The soil was low humic gley associated with reddish brown lattosolic group. For organic treatments, compost was applied to the soil at the rate of 0.5 kg m^{-2} while for inorganic treatments N, P, K fertilizers recommended by the DOA, Sri Lanka were applied as Urea (90 kg/ac) , TSP (22 kg/ac) and MOP (24 kg/ac). Paddy variety Bg 251 was transplanted after 14 days of germination. The pots were maintained at flooded condition. The available N as ammonium, nitrate and P and K of the soil was 8.22 µg/g, 14.614 µg/g, 11.26 µg/g and 22.4 µg/g respectively.

Application of Nostoc sp*. as a biofertilizer*

Wet inoculum of the Nostoc sp*.* was measured and applied at the rate of equivalent dry mass of 1 g kg^{-1} on to the prepared pots according to the treatments mentioned in Table 1.

Application of Liquid fertilizer

The filtered biomass was sonicated, and from that twenty percent of the cell extract of *Nostoc* sp*.* was used as the liquid fertilizer. The first spray was done at 14 days after transplanting. Twenty milliliters of liquid fertilizer were sprayed per plant using a hand sprayer twice a week according to the treatments. Liquid fertilizer was applied for leaves and stems upto four weeks.

Table 1: Treatment Structure

Treatment	Combination
T1	Control (Without any fertilizer)
T2	100 % IF
T3	100 % Compost
T4	50 % IF except N + 2 g *Nostoc* + 20 % *Nostoc* foliar application
T5	50 % Compost + 2 g *Nostoc* + 20 % *Nostoc* foliar application

IF – Inorganic Fertilizer

Plant height and number of leaves were measured at two weeks intervals. Fresh and dry weight of shoot and the grain yield were also measured.

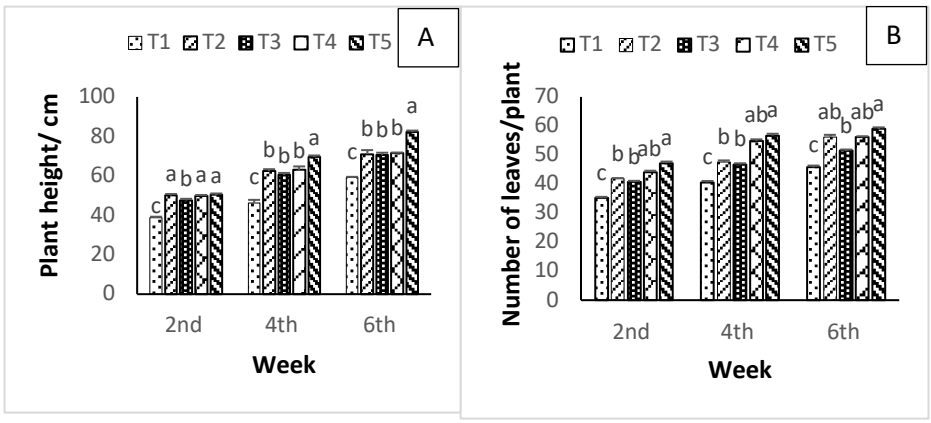
Data analyses were performed by using SAS statistical analytical system (University version) with Duncan mean separation at P=0.05 significance level.

Results and Discussion

The effect of different treatments on plant height shown in figure 1(A). During the 6th week of transplanting, the highest plant height was recorded in T5. Further, it was significantly different from T2, T3 and T4. The lowest plant height was recorded in T1.

Figure 1(B) shows the number of leaves per plant with different treatments. During the 6th week significantly higher number of leaves was recorded in T5. However, it was not significantly different from T2 and T4. At that time the height of the T5 significantly different from T3. The lowest number of leaves was recorded in T1 (control).

Figure 1(C) displaying the fresh weight of the paddy plants in different treatments. The highest fresh weight was recorded in T5 (2 g *Nostoc* sp. + 50 % compost + 20 % foliar spray), however, it was not significantly different from T2 (100 % inorganic). Further, according to the figure 1(D) significantly higher dry weight of shoot was recorded in T5. It was found that the fresh weight of shoots of T2, T4 and T5 were similar, however, the dry weight was significanty higher in T5. It is interesting to note that in T4, the sole source of nitrogen is *Nostoc* sp., which was able to supply the nitrogen equally as inorganic fertilizer. Moreover, the higher growth performace of T5 indicates, that the nutrients supplied by 50% compost enhanced the growth, compared to T4, which is 50% inorganic except nitrogen. Figure 1(E) exhibit the grain yeild of the paddy plants for different treatments. Among all the treatments, grain yield was substantially highest for T5 (2 g *Nostoc* sp. + 50 % compost + 20 % foliar spray) and it was the lowest for T1 (control). T2 (100 % IF) and T4 (2 g *Nostoc sp*. + 50 % IF except N + 20 % foliar spray) also recorded a significantly higher yield except T5. However, the grain yield was not significantly different between T2 and T4.



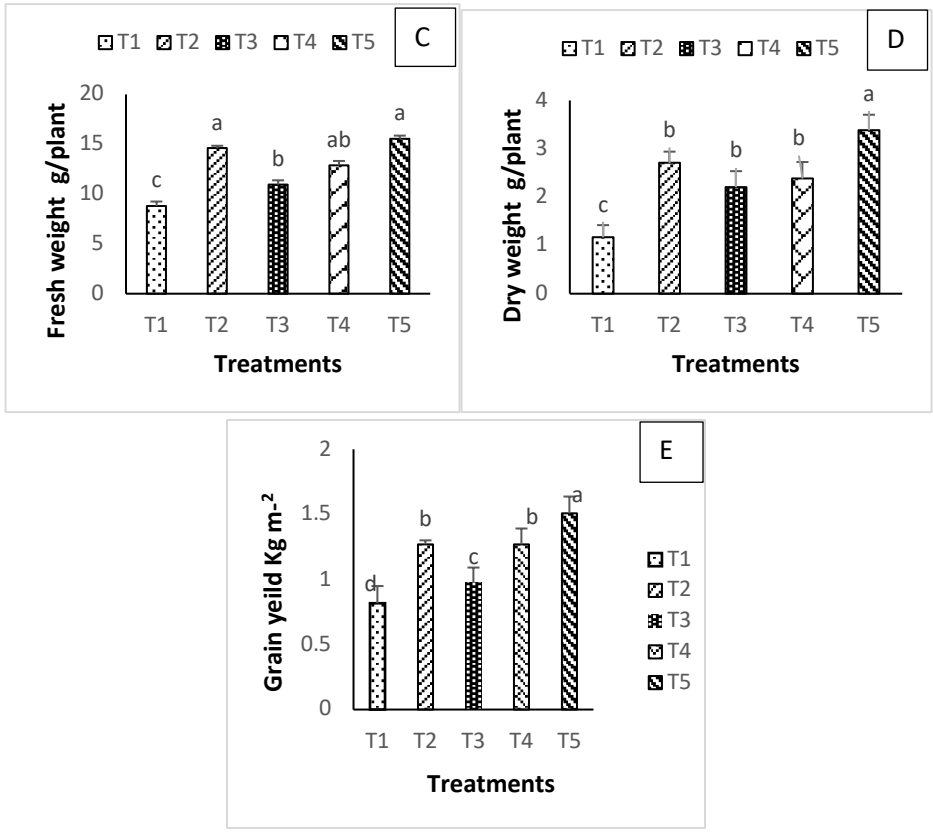


Figure 1. Average plant height (A), Number of leaves / plant (B), fresh weight of shoot (C), Dry weight of shoot (D), Average grain yield of paddy plants grown with different fertilizer treatments:T1-Control, T2-100 % Inorganic Fertilizer (IF), T3-100 % Compost, T4-50 % IF except N + 2 g *Nostoc* sp. + 20 % *Nostoc* sp. foliar application, T5-50 % Compost + 2 g *Nostoc* sp. + 20 % *Nostoc* sp. foliar application. Error bars correspond to the standard error of the mean. Different letters indicate significant differences ($\alpha<0.05$) according to Duncan's multiple range test.

A Previous study reported that incorporation of cyanobacteria species lead to enhance the growth as a result of plant-cyanobacteria association in the rhizosphere [3]. The cyanobacteria species secrete phytohormones which also contribute to improve the crop growth and yield [4]. Another study suggested that half a dose of recommended inorganic fertilizers with *Nostoc commune* was a better option for farmers while considering the cost as well as the quality and quantity of rice [5]. The current study found that combining compost with cyanobacteria species enhances grain yield rather than the sole use of chemical or organic fertilizers.

Conclusion and Recommendations

The results of this pot experiment reveal that the *Nostoc* species is a potential source of nitrogen for paddy cultivation. Further, this cyanobacterium could yield

an equivalent performance as that of DOA recommended inorganic fertilizer, when applied in combination with 50% inorganic fertilizers excluding nitrogen. The combination of compost and *Nostoc* sp. performed the best. Field experiments are required to confirm the effect of *Nostoc* sp. under field conditions. As collection of kitchen waste water of constant quality is difficult, study the use of any other industrial wastewater to grow *Nostoc* sp. is recommended in the future line of research.

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STUDY ON FAT CONTENT AND FATTY ACID PROFILE OF SELECTED COMMERCIALLY AVAILABLE JUNK FOODS IN THE KILINOCHCHI DISTRICT

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Introduction

Junk foods are foods that are easily accessible, usually inexpensive, and low in nutritional content. These foods are higher in calories, salt, and saturated fat and lower in vitamins, iron, calcium, and dietary fiber. Consumption of junk foods in excess amounts leads to various health disorders [2]. A range of fast-food types such as fried foods, carbonated beverages, sweets and chocolates are available for sale in most Sri Lankan food outlets, yet, their nutritional data are scarce. Oil is the major ingredient and the amount of oil that is absorbed by fried foods depends on the type of oil used [4]. Both fried foods and frying oil have an impact on one another and work together to increase the probability of complex chemical events, primarily hydrolysis, oxidation and polymerization in the oil during frying [3]. Trans fats are in a higher percentage in certain kinds of fried foods. The relationship between the dietary consumption of trans fatty acids with increased risk of coronary heart diseases, cancer, obesity and diabetes mellitus has been reported [2]. The World Health Organization (WHO) advises keeping total TFA intake to less than 1% of total calories, or 2.2g per day with a diet of 2000 calories and limiting the consumption of SFAs to less than 15.6 to 22.2 g/day [5].

The present study was designed to quantify the total and trans fatty acid content and fatty acid groups, namely saturated, monounsaturated, and polyunsaturated fatty acid content of junk food products that are heavily consumed by the people in Kilinochchi. The results of this study will be useful to create awareness among people regarding the nutritional quality of junk foods and useful for policy makers in the health sectors.

Materials and Methods

A total of 35 samples were taken from 10 different shops in the Kilinochchi district during March 2022. Roll (n=9), Vadai (n=8), Samosa (n=8), Curry rotti (n=8), and Bonda (n=2) were collected and analyzed. Extraction of fat from samples were carried out by solvent extraction method. Before the solvent extraction the samples were subjected to some pretreatments such as pre-drying of sample and particle size reduction. The samples were cut into small pieces and placed in a hot air oven at 50 ± 5°C until they reached a consistent weight. Then, dried samples were made into powder using a domestic blender. Fat was extracted from

powdered samples by solvent extraction method (Goldfisch method) (VELP Scientifica SER 148) using petroleum ether. The total fat percentage was determined using the following equation.

$$(W2 - W1) / W3 \; x \; 100$$

Where, W1 is Weight of the empty round bottom flask (g), W2 is Weight of the round bottom flask with fat (g) and W3 is Weight of the sample (g).
The fatty acid profile was determined by the following method. Initially, Fatty Acid Methyl Esters (FAMEs) were made by the AOAC 969.33 standard [1] and prepared FAMEs were analyzed by using a gas chromatograph (Agilent Technologies 7890B) which was equipped with a Flame Ionization Detector (FID) and a fused silica capillary column. Identification of Fatty acids was done by comparing their retention time with an appropriate FAMEs standard (Supelco 37 component FAME mix and mixture of trans isomers of linoleic acid).
All experiments were carried out in duplicates. The data were presented as the mean ± standard deviations of the mean using Microsoft Excel 2016. Analysis of Variance (ANOVA) was calculated using the Two factor Completely Randomized Design using Statistical Analysis System (SAS), version 6.0.10. Duncan's multiple range test was used to compare the treatment means among products at $p<0.05$.

Results and discussion

Table 01: Mean value for the total fat content of selected junk foods

Sample No	Roll	Vadai	Samosa	Curry rotti	Bonda
A	20.63 ±1.25^{b}	18.72 ± 1.20^{c}	3.19 ± 0.34^{d}	16.87 ± 0.88^{a}	1.59 ± 0.59^{e}
B	5.40 ±0.24^{f}	20.41 ± 1.05^{cd}	12.19 ± 1.63^{c}	2.00 ± 0.11^{e}	-
C	6.1 ± 0.22^{ef}	17.88 ± 0.68^{c}	15.77 ± 0.77^{b}	12.88 ± 0.98^{b}	-
D	8.17 ± 1.12^{e}	18.42 ± 0.46^{c}	11.20 ± 0.59^{c}	-	-
E	0.81 ± 0.24^{g}	24.24 ± 1.32^{b}	-	10.10 ± 0.68^{c}	-
F	16.22 ± 1.58^{c}	23.41 ± 1.01^{bd}	19.95 ± 1.27^{a}	9.58 ± 0.83^{cf}	-
G	12.10 ± 1.32^{d}	-	11.97 ± 0.16^{c}	5.97 ± 0.62^{d}	15.22 ± 0.79^{b}
H	23.67 ± 1.95^{a}	24.07 ± 2.51^{b}	10.54 ± 0.06^{c}	5.54 ± 1.05^{d}	-
I	15.43 ± 1.07^{c}	-	-	8.13 ± 0.45^{f}	-
J	-	27.74 ± 1.70^{a}	17.18 ± 2.04^{b}	-	-
Mean + Std	12.06 ± 0.63	17.49 ± 0.77	12.75 ± 0.72	8.88 ± 0.31	8.40 ± 0.15
(Min – Max)	(0.81 – 23.67)	(17.88 – 23.41)	(3.19 – 19.95)	(2.00 – 16.87)	(1.59 – 15.22)

Means in each column followed by different superscript letters (a - f) are significantly different (p<0.05).

Table 01 shows the mean values for the total fat content of selected junk foods. It was revealed that vadai contained the highest quantity of fat ($p<0.05$) which is equivalent to 17.49%. The lowest amount, 8.40% was noted in bonda. Samosa contained 12.75% of the second-highest amount of fat, followed by roll (12.06%). The second lowest value of fat was observed in curry rotti, which is equivalent to 8.88%. The total fat content in five different categories of junk food analyzed is in

the order vadai>samosa>roll>curry rotti>bonda. The mean fat percentage of selected junk foods in this study was compared with a previous study conducted in the Colombo district. In comparison to Kilinochchi, the mean roll fat percentage is slightly higher in the Colombo district (12.65>12.06). However, vadai had more fat than the Colombo district (17.49>12.11). We found that the samosa in Colombo City has low fat than the one in Kilinochchi (12.75>10.75). Curry rotti made in the Kilinochchi district has more fat than curry rotti made in Colombo city (8.88>5.65).

Table 02: The mean value of fatty acid composition of selected junk foods

Sample	Roll	Vadai	Samosa	Curry rotti	Bonda
Caproic acid (C6:0)	0.05 ± 0.06	0.08 ± 0.08	0.06 ± 0.08	0.14 ± 0.14	0.11 ± 0.11
Caprylic acid (C8:0)	8.46 ± 0.45	8.63 ± 1.34	8.49 ± 3.08	8.21 ± 0.92	8.35 ± 1.37
Capric acid (C10:0)	6.20 ± 0.45	6.71 ± 0.67	5.87 ± 0.76	5.88 ± 0.37	5.70 ± 1.16
Undecanoic acid (C11:0)	0.21 ± 0.08	0.24 ± 0.14	0.28 ± 0.20	0.39 ± 0.39	0.21 ± 0.00
Lauric acid (C12:0)	46.71 ± 2.05	44.48 ± 4.5	45.21 ± 5.56	44.82 ± 3.78	45.37 ± 4.47
Myristic acid (C14:0)	19.62 ± 0.75	18.90 ± 1.52	19.02 ± 2.01	18.94 ± 1.69	19.05 ± 2.28
Palmitic acid (C16:0)	9.73 ± 2.08	9.93 ± 1.73	9.74 ± 0.96	10.72 ± 0.99	9.43 ± 0.44
Stearic acid (C18:0)	0.03 ± 0.00	0.15 ± 0.21	0.05 ± 0.13	0.16 ± 0.44	0.14 ± 0.19
Total SFA	**91.01 ± 14.87**	**89.12 ± 14.18**	**88.72 ± 14.43**	**89.25 ± 14.28**	**88.36 ± 14.84**
Cis-10 Pentadecanoic acid (C15:1)	0.01 ± 0.36	0.04 ± 0.08	0.03 ± 0.06	0.06 ± 0.10	-
Palmitoleic acid (C16:1)	0.19 ± 0.09	0.29 ± 0.55	0.29 ± 0.28	0.36 ± 0.44	0.22 ± 0.31
Cis-10 Heptadecenoic acid (C17:1)	0.15 ± 0.19	0.06 ± 0.08	0.08 ± 0.17	0.19 ± 0.17	-
Oleic acid (C18:1)	-	2.13 ± 3.02	1.58 ± 2.42	-	-
Total MUFA	**0.35 ± 0.14**	**2.52 ± 1.70**	**1.98 ± 1.32**	**0.61 ± 0.30**	**0.22 ± 0.31**
Linoleic acid (C18:2)	1.41 ± 2.06	1.41 ± 2.34	3.67 ± 7.46	0.97 ± 2.17	2.12 ± 2.99
Total PUFA	**1.41± 2.06**	**1.41 ± 2.34**	**3.67 ± 7.46**	**0.97 ± 2.17**	**2.12 ± 2.99**
Total UFA	**1.76 ± 1.14**	**3.93 ± 1.83**	**5.65 ± 3.59**	**1.58 ± 1.11**	**2.34 ± 2.05**
Linolelaidic acid (C18:2 *trans* 9,12)	6.93 ± 1.89	6.95 ± 4.89	5.63 ± 4.95	8.62 ± 4.57	8.12 ± 3.78
Elaidic acid (C18:1 *trans*)	0.29 ± 0.88	-	-	0.56 ± 1.03	1.19 ± 1.68
Total TFA	**7.22 ± 3.70**	**6.95 ± 4.89**	**5.63 ± 4.95**	**9.17 ± 5.25**	**9.31 ± 4.66**

Thirty-five food samples obtained from 10 shops were analyzed for the fatty acid composition. There was a significant difference among the contents of SFAs, MUFAs, PUFAs and TFAs within and between the selected junk foods which may be due to the re-usage of frying oil. Among food categories, the SFA content of food was higher than the UFA content. Some products consisted of TFAs such as elaidic acid (C18:1 trans) and isomers of linoleic acid. Fourteen different fatty acids were detected in roll, vadai, samosa and curry rotti samples with lauric acid as the dominant SFA. In the bonda sample, twelve distinct fatty acids were found. All junk foods consisted of lauric acid (C12:0) as the predominant SFA (highest as 48.72 ± 0.02%) and myristic acid (C14:0) as the second dominant (>18%) fatty acid. The SFA content varied from 91.26% (Roll) to 87.56% (Vadai). Curry rotti showed the lowest UFA content of 1.37% while the highest UFA quantity was recorded in vadai (9.05%). Most of the fried foods such as rolls, samosa, vadai and bonda contain fairly high amounts of SFA due to the use of palm oil for frying them. Oleic acid (C18:1) and linoleic acid (C18:2) were detected as major MUFA and PUFA, respectively.

Elaidic acid (C18:1 trans-9) and linolelaidic acid (C18:2 trans-6) were identified as the major trans fatty acids in food items analyzed. Elaidic and linolelaidic acids are the major trans fatty acids generated during processing such as partial hydrogenation and frying. Linolelaidic acid (C18:2 trans-6) was detected in all collected samples such as roll, vadai, samosa, curry rotti and bonda. Elaidic acid (C18:1 trans-9) is the predominant trans fatty acid found in most of food samples such as roll, curry rotti and bonda. Both TFA are found in roll and curry rotti samples. The higher percentage of trans fat was found in bonda and a lower value was reported in the roll.

Conclusion

The present study has revealed that the SFAs & TFAs are available in considerable amount of Sri Lankan deep fried food products. Elaidic acid (C18:1) and linolelaidic acid (C18:2) are the main two trans fatty acids detected in the foods tested. WHO suggested that the intake of saturated fats be reduced to less than 10% of total energy intake (15.6-22.2g per day) and trans-fats to be less than 1% of total energy intake (< 2g per 100g of fat). Highest amount of SFA and TFA was detected in vadai which was exceeded the recommendation by WHO. All analyzed samples were consisted high amount of TFA and their amounts surpassed the recommended level given by WHO. So, these foods are unhealthy to eat. Further, the frequent consumption of these foods is associated with the occurrence of various diseases such as; obesity, type-2 diabetes, hypertension and cardiovascular diseases.

Frying oils contain PUFA and trans-fat at high temperatures (>180 °C) for prolonged period of time. Furthermore, the reuse of frying oils many times for frying leads to generation of significant quantities of trans fats. In Sri Lanka,

regulations on oils and fats need to be better aligned with health recommendations as not only TFA but also SFA must be addressed, with standards being more specific with regard to the quality of the fats.

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DIETARY INTAKE OF FLUORIDE FROM THE CONSUMPTION OF BLACK TEA IN CKDu PREVALENT AREAS, SRI LANKA

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Introduction

Chronic kidney disease of uncertain etiology (CKDu) is characterized without any recognized risk factors such as hypertension or diabetes for chronic kidney disease (CKD). CKDu was observed in the mid-1990s among Sri Lankan farmers in the North Central Region. However, the disease has spread to other farming areas during the last two decades. The exact causative factors behind CKDu in the dry zone rural agricultural areas of Sri Lanka is still unknown. It is determined that there are multiple factors that contribute to the development of CKDu, including genetic factors, nutritional status and dehydration, susceptibility to nephrotoxins in the environment, and lifestyle variables. Chronic exposure of kidney membrane to Hofmeister-type anions such as fluoride are considered as a significant risk factor for chronic kidney disease of uncertain etiology in the regions of dry agricultural zone in Sri Lanka. Fluoride may impact renal tissues because of its Hofmeister type protein denaturing mechanism and denature the protein of the kidney membrane [1].

However, most studies consider a single exposure pathway such as groundwater and a lack of attention is given to understanding the exposure through beverages other than drinking water such as tea, which is a common and frequent beverage in the rural population of Sri Lanka. The tea plant roots have the ability to hyper accumulate fluoride in the soil and fluoride content high in older tea leaves. When tea leaves are brewing, at least 75% of the fluoride content in tea leaves are extracted into boiling water [2]. Black tea is the most common beverage and a high rate of consumption is observed among labour intensive workforce in Sri Lanka. Traditional tea drinking behaviors may enhance the fluoride intake in the population. In order to ensure food safety and consumer health, it is essential to routinely evaluate these samples for fluoride. Excessive fluoride has detrimental effects on the growth of the industry and poses health hazards to consumers. Hence, the objective of present study was to investigate the concentration of fluoride in black tea and tea infusion consumed in households where CKDu mostly affected regions in Sri Lanka and to assess the dietary exposure risk.

Methodology

Study site and sample collection

Twenty one black tea samples were collected from random households in CKDu endemic Dehiaththakandiya area (Latitude: 7.67128; Longitude: 81.046484), Sri Lanka.

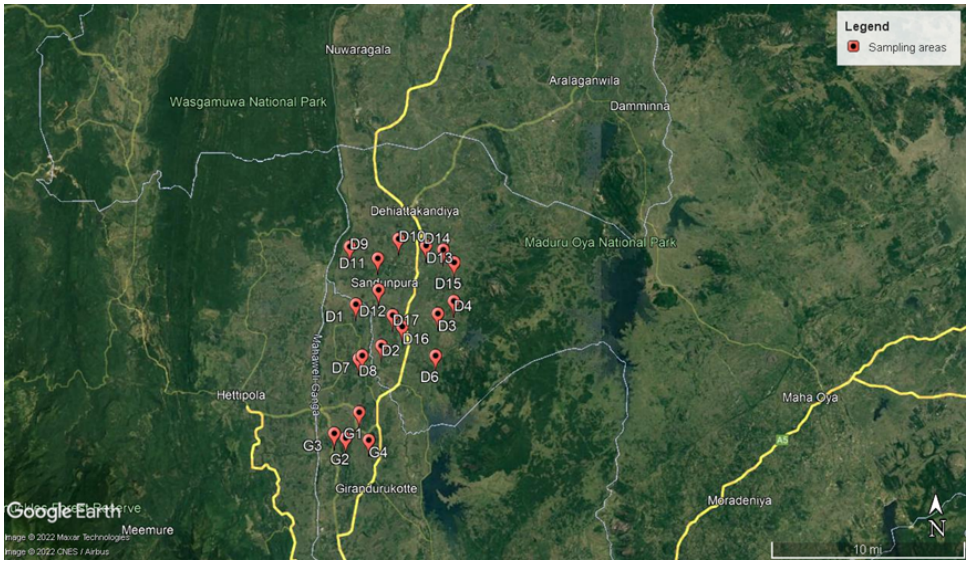

Figure1. Sample collecting locations in Dehiaththakandiya

Determination of fluoride concentration in black tea samples

Impoverished farming communities tend to consume low-quality tea which is available in the market (known as dust tea, without any brand or label). Hence total fluoride content in tea samples was determined.

Alkaline digestion of tea samples

0.5 g of black tea and volume of 2 mL 50 w/v prepared KOH solution were added into the nickel crucible, then heated at 100 °C for 30 min on a hot plate. Then the tea samples were ashed at 300 °C using a muffle furnace and the heating temperature was increased up to 600°C by the rate 200 °C/ 1 hr. Finally, the samples were remained left in the furnace for further 30 min and allowed to cool [3]. Then samples were added to volumetric flask, made up the volume to 50 mL and they were filtered using 0.45 µm cellulose nitrate membrane filters.

Analytical procedure

Black tea samples were analysed for total fluoride concentration using a Metrohm 930 Compact Ion Chromatograph Flex with conductivity detector (Metrohm A Supp 5–250/4.0 column). The eluent was 3.19 m mol L^{-1} $NaCO_3$ and 1.0 m mol L^{-1} $NaHCO_3$ with a flow rate (0.7 mL min^{-1}); running time (30 min) with 20 µL volume of injection. Instrument was calibrated using 0.25, 0.5, 1.0, and 2.0, 3.0 mg L^{-1}

fluoride solution, prepared using Fisher 100 mg L^{-1} standard stock solution of fluoride.

Fluoride concentration in black tea infusion

Preparation of tea infusion

Selected black tea samples from CKDu endemic (n=5) were used to determine the fluoride concentration leached into tea infusions prepared from deionized water. Total weight of black tea (2.0 g) was added in to a glass beaker with 200 mL of boiling deionized water (1% w/v) and infusion was collected after brewing for 5, 10, 20, 30, 60, 90, and 120 min. In order to assess the influence of brewing time. During all the brewing time the beaker was heated on a hot plate with a temperature between 80 and 85 °C. For each time duration, 10 mL liquor was pipetted out and filtered using a 0.45 µm cellulose nitrate membrane filter.

Analytical procedure

Fluoride content in the black tea infusion filtrate was analyzed using Fluoride Combination Ion-Selective Electrode (ISE) HI4110. For that cooled tea infusion was mixed with 1:1 (v/v) TISABII.

Dietary exposure of fluoride from black tea and risk assessment

The value of chronic daily intake (CDI) of fluoride through the black tea consumption was calculated using the following equation (equation 01).

$$CDI=(C\times DI\times EF\times ED)/(BW\times AT) \quad \text{(Equation 01)}$$

Where, C is the mean concentration of fluoride in black tea infusion (mg L^{-1}), DI consider as average daily intake rate of tea (L per day), EF is the frequency of exposure (day(s) per year), ED is the duration of exposure (year), BW is body weight (kg), and AT is the averaging time (days). The average body weight of adults in the dry zone of Sri Lanka is considered as 56.4 kg.

Hazard Quotient was calculated (HQ) was calculated using the equation 02.

$$HQ=CDI/RfD \quad \text{(Equation 02)}$$

Where HQ is Hazard Quotient and RfD is Reference dose. When the HQ is higher than 1, the chronic daily intake of fluoride exceeds the RfD and there may be a risk.

Statistical analysis

Statistical analysis was carried out by using SPSS software for one way ANOVA. When p was ≤ 0.05, results were considered statistically significant.

Results and Discussion

Fluoride content in black tea

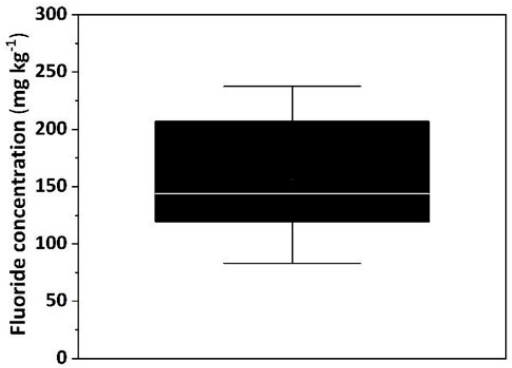

Figure 2. Box and Whisker plot for fluoride in black tea samples

Mean, median and maximum level of fluoride in collected black tea samples are depicted in Figure 2. The total fluoride concentration in black tea samples collected from CKDu affected Dehiattekandiya area ranged from 83-237.5 mg kg^{-1} with a mean of 157.55±47.98 mg kg^{-1}. Fluoride concentration in black tea samples of this study is more similar to the findings of [4] and [5] where total fluoride concentrations were in the range of 37.1-225 mg kg^{-1} and 47.05-291.98 mg kg^{-1}. Fluoride content in tea was increased with the age of tea plant leaves and it might be considered as one of the main factors which affect the variation of fluoride level in black tea sample [2].

Fluoride in tea infusions

Selected black tea samples (n=5) were used to determine the fluoride concentration leached into tea infusions prepared using deionized water. As depicted in Figure 3, mean fluoride concentration in the samples taken from 5, 10, 30, 60, 90- and 120-min intervals were 1.47±0.43, 1.53±0.46, 1.66±0.48, 1.84±0.66, 1.92±0.71, and 2.09±0.88 mg L^{-1} respectively. Moreover, with the increasing of infused time the concentration of fluoride in tea infusions prepared from CKDu area samples also demonstrated a significant increment ($p<0.05$) and it was comparable with the findings of [2] stated that Increasing the brewing time extended the reactions between tea leaf grains and hot water and contribute to a higher level of fluoride ion in tea infusion.

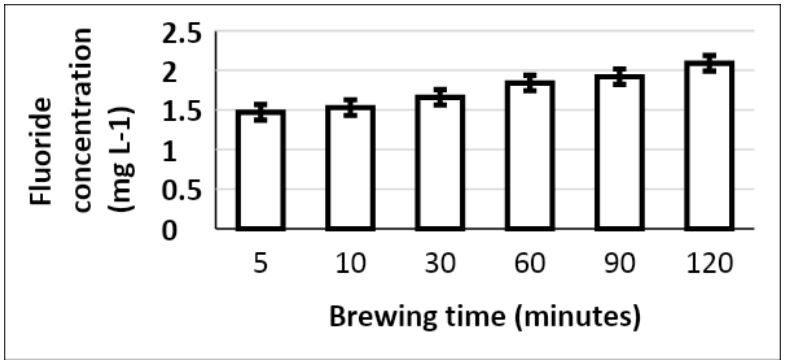

Figure 3. Fluoride concentration (mg L^{-1}) for different brewing times

Dietary exposure of fluoride through black tea and risk assessment

The chronic daily intake value of fluoride from the consumption of tea infusion was calculated considering the average volume tea consumption 1.0 L per day and the average body weight of adults in the region of dry zone Sri Lanka is 56.4 kg. The calculated chronic daily intake of fluoride from tea used in CKDu endemic areas was 0.026 mg/day/kg body weight for 5 minutes of infusion and 0.037 mg/day/kg body weight for 120 minutes of tea infusion (Table 1).

Table 1. Chronic Daily Intake values and Risk Assessment

Tea infusion	Fluoride concentration (mg L^{-1})	CDI (mg/day/ body weight)	HQ
5 min	**1.47**	**0.026**	**0.52**
120 min	**2.09**	**0.037**	**0.74**

These values did not exceed the estimated adequate daily intake value of fluoride 0.035-0.067 mg/day/kg body weight for the communities live in CKDu affected regions in Sri Lanka

Considering the fluoride risk assessment from tea, the HQ values of 5 min and 120 min tea infusions were 0.52, and 0.74 respectively. When HQ value is higher than 1.00, the exposure assessment of fluoride exceeds the RfD value and there may be a potential risk factor for adverse health effects for people living in CKDu endemic areas in Sri Lanka.

Conclusions and Recommendations

The chronic daily intake of fluoride only from tea infusion did not exceed the critical range. However, CKDu endemic population use groundwater already contains elevated levels of fluoride to prepare tea infusions and brewed nearly two hours as their traditional tea preparation behaviors. This groundwater would raise the fluoride content of tea if they were used to brew it. Aggregated chronic daily intake value of fluoride from both tea and drinking water should be considered for further studies and it may be exceeded the critical range. In addition to black tea infusions, dietary intake of fluoride from other food and

beverage sources commonly practiced in CKDu endemic areas need to be taken into consideration.

Acknowledgements
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EFFECT OF *Chlorella* sp. AND COMBINATIONS OF SELECTED NUTRIENT SOURCES ON GROWTH AND YIELD OF ONION (*Allium cepa* L.)

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Introduction

The worldwide increase in human population leads to an increase in the demand for food, which must be supplied by increasing crop production. One of the best options to increase crop production is integrated nutrient management, which incorporates the utilization of different sources of plant nutrients. Compost is an organic component of an integrated nutrient supply system, which enhances soil health, improves macro and micronutrient availability, and increases crop productivity [1]. Biofertilizers are also a key component in integrated nutrient management. *Chlorella* is a green alga rich in macro and micronutrients [2], however, a huge amount of water and nutrients are essential for cultivating *Chlorella.* The effluent produced in the dairy industry contains a high amount of nutrients and therefore could be used as a growth medium for the cultivation of *Chlorella.* In this context, this study was conducted to check the potential of cultivating *Chlorella* sp. in dairy industry wastewater and to find the potential effect of different nutrient sources (inorganic, compost, and *Chlorella*) and their combinations on the growth and yield of onion (*Allium cepa* L.).

Materials and Methods

Culturing of Chlorella sp.

Stock culture of microalgae was centrifuged at 3000 rpm for 20 min to provide seed microalgae cultures with a 0.2 optical density (OD). 250 L open raceway reactor was used to cultivate *Chlorella* sp. in dairy wastewater medium. Two open raceway reactors were used, each was filled with 135 L of wastewater equally and then inoculated with seed cultures of *Chlorella* sp. A paddle wheel was incorporated into the raceway reactor to facilitate the continuous mixing of the cultures and to prevent the settling of microalgae. The raceway reactors were continuously illuminated using Halogen lamps to provide light for algal photosynthesis. The photosynthetically active radiation (PAR) incident on the surface of the reactor was 200 µmol $m^{-2}s^{-1}$. The experiment was carried out at 28-30 °C at ambient laboratory conditions. The reactor was continuously operated for 14 days.

Harvesting and biomass separation of Chlorella
After two weeks of cultivation, microalgae biomass was separated from dairy wastewater medium by sedimentation technique.

Analysis of Organic Sources
Analyzed properties of *Chlorella* biomass were here analyzed. pH and EC were measured using a pH meter and an Electrical conductivity meter respectively. Total nitrogen was estimated according to Kjeldhal method [4]. Phosphorus and potassium contents were determined using the Vanadomolybdate method [5] and flame photometer [6] respectively.
Field experiments on *Allium cepa* L. *to evaluate the effect of nutrient sources*
A field experiment on onion (*Allium cepa* L. - Vethalam variety) was conducted in a complete randomized block design. There were eight treatments (T1 - (100 % inorganic fertilizer), T2 - (Compost 9 t/ha), T3 - (Compost 4.5 t/ha), T4 - (25 % inorganic fertilizer+ Compost 4.5 t/ha), T5 - (25 % inorganic fertilizer + Compost 4.5 t/ha + 15 % *Chlorella* foliar spray), T6 - (Compost 4.5 t/ha + 15 % *Chlorella* foliar spray), T7 - (Compost 4.5 t/ha + 20 % *Chlorella* foliar spray), T8 - (Control)) with three replicates. All other management practices were done as per the recommendation of the DOA.

Fertilizer Application
For organic treatments, both compost and *Chlorella* bio-fertilizer were applied. *Chlorella* bio-fertilizer was applied as a foliar spray and the compost was applied to the soil according to the treatment before planting. For inorganic treatments, N, P, and K fertilizers were applied at the DOA recommended rates for onion as Urea, TSP, and MOP. According to the treatments, inorganic fertilizers were applied to as basal and top dressings. The onion was treated with two different percentages (15 % and 20 %.) of *Chlorella* foliar sprays.
Data Collection and Statistical Analysis
Data collection was done on both growth parameters (plant height, leaf number) and yield parameters (total bulb yield, number of bulbs per plant, and bulb diameter). Data analysis was done by using a statistical analytical system (University version) with Duncan's Multiple Range Test (DMRT) at p=0.05.

Results and Discussion
In this study, 424.71 g of *Chlorella* dried biomass was obtained from 540 L dairy effluent under ambient laboratory conditions. In this study, the total nitrogen, total phosphorous and total potassium contents (w/w %) of harvested dried microalgae were 2.5 %, 2.8 %, and 1.96 %, respectively.

Table 1. Plant height, leaf number and nutrient application

Treatments	Plant Height(cm)		Number of Leaves	
	2nd WAP	4th WAP	2nd WAP	4th WAP
T1	$25^{a} \pm 0.3$	$28.3^{a} \pm 1$	$22^{a} \pm 2$	$29^{a} \pm 2.6$
T2	$24.3^{a} \pm 0.8$	$26.7^{ab} \pm 1.2$	$20^{ab} \pm 1.4$	$26.7^{ab} \pm 0.8$
T3	$26.3^{a} \pm 0.3$	$28.7^{ab} \pm 0.6$	$16.7^{c} \pm 0.3$	$28.3^{ab} \pm 2$
T4	$24^{a} \pm 2.0$	$27^{ab} \pm 1.52$	$14.7^{cd} \pm 1.2$	$20.3^{cd} \pm 1.7$
T5	$23.7^{a} \pm 0.3$	$28^{ab} \pm 0.3$	$18^{bc} \pm 0.5$	$23^{bc} \pm 1.1$
T6	$22.7^{a} \pm 0.8$	$25.3^{bc} \pm 0.8$	$14.7^{cd} \pm 0.8$	$23^{bc} \pm 1.2$
T7	$24.3^{a} \pm 0.5$	$27.3^{ab} \pm 0$	$18.7^{ab} \pm 2.1$	$24^{ab} \pm 2.3$
T8	$18.3^{b} \pm 1.2$	$21.3^{c} \pm 0.8$	$11.3^{d} \pm 1.7$	$14.3^{e} \pm 2$

WAP- weeks after planting, T1 - 100 % inorganic (DOA), T2 - Compost 9 t/ha, T3 - Compost 4.5 t/ha, T4 - 25 % inorganic (DOA) + Compost 4.5 t/ha, T5 - 25 % inorganic (DOA) + Compost 4.5 t/ha + 15 % *Chlorella* foliar spray, T6 - Compost 4.5 t/ha + 15 % *Chlorella* foliar spray, T7 - Compost 4.5 t/ha + 20 % *Chlorella* foliar spray, T8 – Control. The same letters within columns are not statistically different according to the DMRT at P=0.05

Table 2. Nutrient application rate according to the treatments

	Nutrient Application		
Treatments	N Kg/ha	P Kg/ha	K Kg/ha
T1	89.7	46	45
T2	180	90	180
T3	90	45	90
T4	112.4	56.5	101.3
T5	116.9	61.6	104.8
T6	94.5	50.1	93.8
T7	94.9	50.5	93.8
T8	0	0	0

T1 - 100 % inorganic (DOA), T2 - Compost 9 t/ha, T3 - Compost 4.5 t/ha, T4 - 25 % inorganic (DOA) + Compost 4.5 t/ha, T5 - 25 % inorganic (DOA) + Compost 4.5 t/ha + 15 % *Chlorella* foliar spray, T6 - Compost 4.5 t/ha + 15 % *Chlorella* foliar spray, T7 - Compost 4.5 t/ha + 20 % *Chlorella* foliar spray, T8 – Control.

The effect of different treatments on plant height and number of leaves is shown in Table 01. In the second week after planting, the highest plant height was recorded in T3 (Compost 4.5 t/ha), however, it was not significantly different from all others except T8 (control). In the fourth week after transplanting, the highest height was observed in T1 (100 % inorganic), however there was no any significant difference among other treatments except T6 (Compost 4.5 t/ha + 15 % *Chlorella* foliar), and T8 (control). The lowest height was recorded in T8 (control), it was not significantly different from T6 (Compost 4.5 t/ha + 15% *Chlorella* foliar). In the second week after planting, the highest number of leaves per plant was recorded in T1 (100 % inorganic fertilizer), however there was no significant difference in the number of leaves among the treatments of T1, T2 (Compost 9 t/ha) and T7 (Compost 4.5 t/ha +20 % *Chlorella* foliar spray). A lower number of leaves per plant was recorded in T8 (control), however it was not significantly different from the treatments, T4 (25 % inorganic fertilizer + Compost 4.5 t/ha) and T6 (Compost

4.5 t/ha + 15 % *Chlorella* foliar). In the fourth week after planting the highest number of leaves per plant was recorded in T1 (100 % inorganic fertilizer), however there was no significant difference among the treatments of T1, T2 (Compost 9 t/ha), T3 (Compost 4.5 t/ha) and T7 (Compost 4.5 t/ha +20 % *Chlorella* foliar spray). The lower number of leaves per plant was observed in T8 (control).

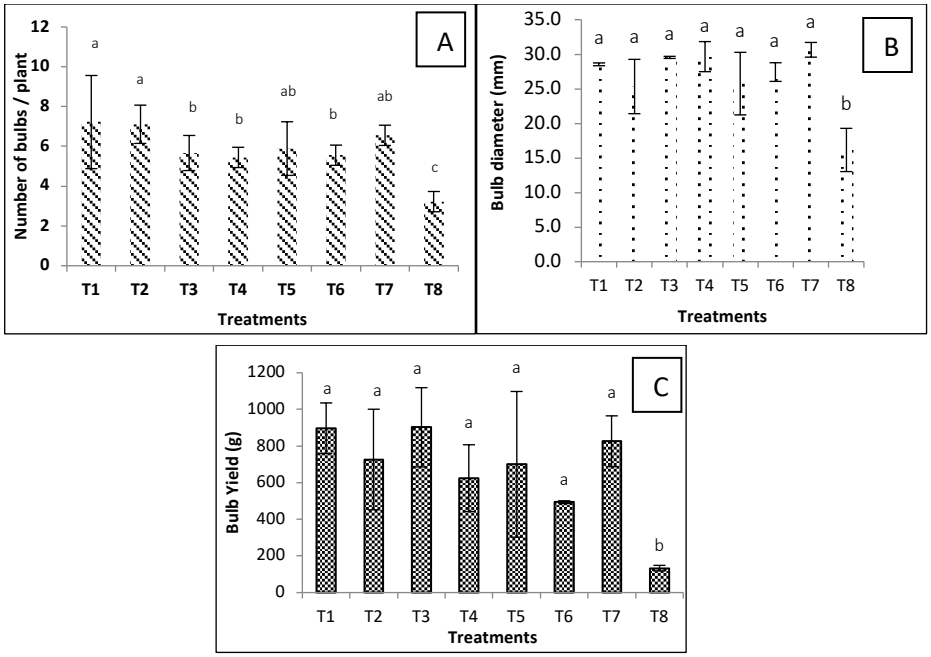

Figure 1. Variation in number of bulbs per plant (A), bulb diameter (B) and bulb yield (C) with treatments. T1 - 100 % inorganic (DOA), T2 - Compost 9 t/ha, T3 - Compost 4.5 t/ha, T4 - 25 % inorganic (DOA) + Compost 4.5 t/ha, T5 - 25 % inorganic (DOA) + Compost 4.5 t/ha + 15 % *Chlorella* foliar spray, T6 - Compost 4.5 t/ha + 15 % *Chlorella* foliar spray, T7 - Compost 4.5 t/ha + 20 % *Chlorella* foliar spray, T8 – Control. The same letters within columns are not statistically different according to DMRT at P=0.05

Figure 1 displays variation in the number of bulbs per plant, bulb yield, and bulb diameter with different treatments. The high number of bulbs per plant (7 bulbs) was observed in both T1 (100 % Inorganic fertilizer) and T2 (Compost 9 t/ha). The highest bulb diameter (3.1 cm) was recorded in T7 (Compost 4.5 t/ha + 20 % Chlorella foliar spray). However, there was no any significant difference observed among other treatments except T8 (Control). The average yield for treatments ranged from 113.3 g (T8 - control) to 902.4 g (T3 - Compost 4.5 t/ha). There was no significant difference in yield among the treatments except T8 (Control).

According to Table 02 nutrient supply through the 4.5 t/ha compost was able to supply the required nutrients almost the same (N and P) or above (K) the Agricultural Department recommendations (N-89.7Kg/ha, P-46Kg/ha, K-45Kg/ha), hence the effect of *Chlorella* foliar application in combination with

compost did not provide any significant positive effect in this experiment. Therefore, during future studies amount of compost application should be reduced to find the effect of *Chlorella* foliar application.

Conclusion and Recommendations

In raceway reactors under ambient laboratory settings, a *Chlorella* mass culture experiment showed that it is possible to obtain 0.78 gL^{-1} dry biomass yield from dairy effluent without any additional nutrients. *Chlorella* sp. cultivated in dairy wastewater has a nutritional content of 2.5 %t nitrogen, 2.8 %phosphorus, and 1.96 %potassium, and it has the potential to be a source of nutrients. A good alternate solution to the problem of water scarcity is to grow *Chlorella* in dairy waste rather than in clean water. In this study, T3 (Compost 4.5 t/ha) had the highest bulb yield, however there was no significant difference among all other treatments except T8 (Control). This research study proved that the organic fertilizer, combination of organic fertilizer with biofertilizer, and #Integrated Plant Nutrient System (IPNS) combination express similar yield like sole to inorganic fertilizer.

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SCREENING OF BLACKGRAM (*Vigna mungo.* L) 4 GERMPLASMS AGAINST POWDERY MILDEW AND YELLOW MOSAIC VIRUS DISEASES

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Introduction

Black gram is one of the primary grain legumes grown in Sri Lanka's dry and intermediate zones under rain-fed agriculture. It can be grown in low-humidity, fertile environments. The blackgram production is restricted by a number of abiotic and biotic factors such as unfavorable climate, nutritional imbalance, pest and diseases caused by fungi, bacteria, viruses, nematodes. The main reason for low yield is, susceptibility of the crop to insects, weeds and diseases. Generally, we can find powdery mildew, yellow mosaic virus (YMV), anthracnose, leaf spot, blight, root rot, rust, dry root rot, leaf crinkle disease, leaf curl, ascochyta leaf spot and bacterial blight disease in blackgram (Pratap *et al.,*2020). Powdery mildew disease and yellow mosaic virus can be considered as major diseases of black gram in Sri Lanka (Karthikeyan *et al., 2014)*. Powdery mildew is caused by *Erysiphe polygoni* and it occurs at lager stages of crop causing yield loss of 20 % (Singh, 1995). Plant viral diseases cause serious economic losses in many grain crops by reducing seed yield and quality of seeds (Schreinemahers *et al.*, 2015). Among the number of viral diseases, the yellow mosaic virus is the most serious disease and major bottle neck for the production of blackgram. The YMV is transmitted by whitefly *Bemisia tabaci* (Pavishna, 2019). This study mainly focuses on identifying the resistant/ tolerant germplasms for Powdery mildew disease and Yellow Mosaic Virus disease of Blackgram to use in breeding programmes.

Materials and Methods

The field experiment was conducted at the Field Crops Research and Development Institute (FCRDI), Mahailuppallama. Experiment was carried out during the yala season from May to August. Seeds of 4 germplasms of blackgram which were developed by the FCRDI were used for the experiment with 2 locally check varieties; MI 01 and MI BG 04. The data were collected from middle 3 ridges and other 2 ridges were used as borders. Disease Severity Index (DSI) and Disease Incidence (DI) were calculated. Randomized Complete Block Design (RCBD) with 3 replicates were used as experimental design.

Disease Incidence (DI)

DI was calculated as the proportion of infected plants per plot and expressed as a percentage.

$$DI = \frac{\text{Number of plants affected}}{\text{Total number of plants}}$$

Disease Severity Index (DSI)

DSI was calculated by using the disease rating scale on a 0-9 scale for the selected diseases. The scale was used to measure the DSI of YMV based on Mohan *et* al., (2014) and the scale for PM was based on Khandappagol and Rangaiah, (2019). The disease severity index (DSI) for each disease was calculated according to the formula mentioned below.

$$DSI = \frac{\text{Sum of numerical rating scale}}{\text{Number of observations} \times \text{maximum rating scale}}$$

Table 1. Disease Rating Scale (0-9) for powdery mildew, yellow mosaic virus based on Disease Severity Index

Disease rating scale	Percent infection
0	No plants showing any symptoms
1	Less than 1% plants exhibiting symptoms
3	1-10% plants exhibiting symptoms
5	11-20% plants exhibiting symptoms
7	21-50% plants infection
9	50% and more plants exhibiting symptoms

According to the DSI scale, Blackgram germplasms were categorized into the level of resistance or susceptibility.

0 = Highly Resistant (HR)
1-10 = Resistant (R)
10-20 = Moderately Resistant (MR)
20-30 = Moderately Susceptible (MS)
30- 50 = Susceptible(S)
>50 =Highly Susceptible (HS)

Results and discussion

DI Assessment to yellow mosaic virus during yala season

The disease incidence varied from 31.6 % - 94.4 % in 8 weeks after planting and showed significant variation among genotypes ($p<0.05$). Germplasm 17-162

showed the lowest disease incidence (31.6 %) than the local check variety MI BG 04. Significantly highest DI has been shown by the germplasm 17- 040 (94.4%) followed by germplasm 17-148 (91.4%) also indicate much higher disease incidence than the local check variety MI 01. Germplasm 17- 051 (82.7%) was showed much higher DI. But it was not higher than local check variety MI 01 (91.1%). Out of 6 blackgram genotypes, germplasm 17-162 showed the lowest disease incidence during the observation period. That incidence is lower than local check variety MI BG 04. All these germplasms are significant with locally checked varieties, MI 01 and MI BG 04. Among these 2 varieties MI 05 is susceptible for YMV and MI BG 04 is resistance for YMV. In contrast, significantly highest DI has been shown by the germplasm 17- 040 followed by germplasm 17-148 also indicate much higher disease incidence than the local check variety MI 01. Germplasm 17- 051 was showed much higher DI. But it was not higher than local check variety MI 01. None of the four germplasms tested were found to be free of infection.

Table 2. Disease Incidence of different Blackgram genotypes for Yellow Mosaic Virus

Genotypes	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP
17-148	11.76±0.21^{ab*}	33.1±0.52^{a}	53.2±0.21^{ab}	87.1±0.12^{a}	91.4±0.21^{ab}
17-162	4.3±0.34^{b}	5.2±0.23^{b}	16.2±0.12^{d}	27.6±0.18^{b}	31.6±0.03^{c}
17-051	6.9±0.37^{ab}	18.3±0.12^{ab}	41.6±0.38^{bc}	78.6±0.21^{a}	82.7±0.01^{b}
17-040	13.7±0.03^{a}	34.1±0.03^{a}	60.0±0.41^{a}	87.8±0.28^{a}	94.4 ±0.32^{a}
MI 01	13.1±0.21^{ab}	29.2±0.32^{a}	45.7±0.23^{b}	87.0±0.08^{a}	91.1±0.21^{ab}
MI BG 04	8.4±0.32^{ab}	15.2±0.31^{ab}	30.3±0.21^{c}	34.2±0.08^{b}	37.8±0.22^{c}
F- Value	*1.78*	*2.74*	*13.92*	*76.82*	*56.08*
P- Value	*0.20*	*0.07*	*0.02*	*0.01*	*0.01*
C.V (%)	*46.52*	*46.01*	*15.93*	*7.09*	*8.01*

**The means followed by the same letter in each column are not significantly different by the Duncan's multiple range test at 5% level of probability.*

DSI Assessment YMV during yala season

According Kundu et al., (2022) germplasm 17-162 was less severely affected by the YMV out of the 6 blackgram genotypes, and it was ranged as moderately resistant (10–20%). This germplasm showed a lower DSI value than the local check variety MI BG 04. MI BG 04 was also classified as moderately resistant. All other blackgram genotypes (germplasm 17-040, germplasm 17-148, and germplasm 17-051) were highly susceptible to YMV since they were in the >50 DSI range. MI 01 was also classified as highly susceptible.

Table 3. Disease Severities of Different Blackgram Genotypes to Yellow Mosaic Virus

Genotypes	6 WAP	7 WAP	8 WAP	Reaction
17-148	22.0±0.01^{ab*}	62.1±0.23^{ab}	76.3±0.49^{a}	HS
17-162	3.7±0.12^{c}	10.0±0.32^{c}	14.9±0.48^{c}	MR
17-051	16.6±0.12^{b}	53.2±0.31^{b}	65.7±0.45^{b}	HS
17-040	26.0±0.32^{a}	67.0±0.03^{a}	82.5±0.84^{a}	HS
MI 01	18.5±0.38^{b}	60.9±0.86^{ab}	75.1±0.45^{a}	HS
MI BG 04	8.5±0.83^{c}	15.7±0.84^{c}	17.5±0.42^{c}	MR
F- Value	11.65	39.45	91.99	
P- Value	0.0004	< .0001	< .0001	
C.V (%)	23.13	13.12	8.50	

Note: WAP = Weeks after Planting, HR= highly resistant, R= resistant, MR=moderately resistant, MS= moderately susceptible, S= susceptible, HS= highly susceptible.

**The means followed by the same letter in each column are not significantly different by the Duncan's multiple range test at 5% level of probability.*

DI Assessment to PM during yala season

The disease incidence of different Blackgram genotypes against PM disease according to their symptomology. There was no significance difference ($p>0.05$) between the genotypes in six weeks after planting and germplasm 17-040 (80.7%), germplasm 17- 051 (80.3%), germplasm 17- 148 (77.1%), germplasm 17- 162 (76.3%) showed the highest disease incidence than the local check varieties.

Table 4. Disease Incidence of Different Blackgram Genotypes to Powdery Mildew

Genotypes	6 WAP	7 WAP	8 WAP	9 WAP	10 WAP
17-148	77.1±0.60^{a*}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}
17-162	76.3±0.46^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}
17-051	80.3±0.08^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}
17-040	80.7±0.03^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}
MI 01	75.4±0.54^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}
MI BG 04	70.9±0.76^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}	100.0±0.01^{a}
F- Value	0.70	-	-	-	-
P- Value	0.6752	-	-	-	-
C.V(%)	8.29	0	0	0	0

Note: WAP = Weeks after Planting*The means followed by the same letter in each column are not significantly different by the Duncan's multiple range test at 5% level of probability.

**The means followed by the same letter in each column are not significantly different by the Duncan's multiple range test at 5% level of probability.*

DSI Assessment for powdery mildew

Disease severities changes of 6 genotypes are presented in table 5. There was no significant different($p>0.05$) between the genotypes within observation period. All the germplasms (17-051, 17-040, 17-162, 17- 148) showed higher disease severity than the local check variety MI 01 and MI BG 04 and these germplasms were ranged as susceptible for powdery mildew. Local check variety MI 01 and MI BG 04 also categorized as susceptible.

Table 5. Disease Severities of Different Blackgram Genotypes to Powdery Mildew

Genotypes	7 WAP	8 WAP	9 WAP	10 WAP	Reaction
17-148	34.7±0.51^{a*}	36.1±0.23^{a}	55.1±0.15^{a}	67.3±0.31^{a}	S
17-162	35.1±0.53^{a}	37.2±0.24^{a}	56.7±0.29^{a}	67.4±0.39^{a}	S
17-051	37.5±0.38^{a}	39.0±0.43^{a}	59.6±0.54^{a}	68.8±0.41^{a}	S
17-040	37.3±0.28^{a}	39.0±0.34^{a}	56.8±0.29^{a}	68.5±0.42^{a}	S
MI 01	32.4±0.31^{a}	34.5±0.25^{a}	54.9±0.23^{a}	66.2±0.48^{a}	S
MI BG 04	32.2±0.21^{a}	34.2±0.52^{a}	49.5±0.81^{a}	64.3±0.56^{a}	S
F- Value	0.71	0.72	0.55	1.18	
P- Value	0.6640	0.6572	0.7832	0.3927	
C.V(%)	11.29	10.02	12.30	3.82	

Note: WAP = Weeks after Planting; HR= highly resistant, R= resistant, MR=moderately resistant, MS= moderately susceptible, S= susceptible, HS= highly susceptible.

**The means followed by the same letter in each column are not significantly different by the Duncan's multiple range test at 5% level of probability.*

Conclusions and Recommendations

Germplasm 17-162 was found to be as moderately resistant for yellow mosaic virus showing less symptoms with the significant difference ($P<0.05$) with all the tested germplasms and susceptible check except resistant check MIBG 04. Other tested germplasms, germplasm 17-040, 17-148 and 17- 051 were found to be highly susceptible to yellow mosaic virus under field screening. Results further concluded that, all the germplasms including check varieties were susceptible to powdery mildew disease under field screening. None of blackgram genotype was found resistant and highly resistant for both diseases. Germplasm 17-162 is an important source to use in yellow mosaic virus resistant breeding programme. Even though, all prior to exploitation in resistance breeding program, the genetic basis of their resistance would be determined. It is very essential to understand the nature of resistance before developing improved varieties.

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ASCERTAINING A SUITABLE ACIDITY REGULATOR FOR YELLOW PASSION FRUIT (*Passiflora edulis* f. *flavicarpa*) DRINKING YOGHURT

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Introduction

Production and consumption of fermented dairy products are increasing day by day due to their flavor and high nutrition. The current trend is fortifying dairy products with fruits or by-products of fruits, intending to deliver healthy food. The blend of yoghurt and fruit will provide high-quality protein, vitamins, minerals and fatty acids [1]. Passion fruit (*Passiflora edulis* f. *flavicarpa*) is consumed as fresh fruit and incorporated in juice, jam, jelly and ice cream productions. Citric and malic acid are the dominant acids present in passion fruit and is responsible for their high acidity. The titratable acidity by means of citric acid in passion fruit juice is 1.370.0 per 100 g. However, this high acidic nature becomes a barrier when applied to certain products. Coagulation of the drinking yoghurt was observed when passion fruit was incorporated and it ultimately deteriorated the sensorial and physicochemical properties of the product. The citric acid content can be decreased by several methods, thus increasing the pH. The purpose of adding acidity regulators to a food product is important in regulating the pH in order to make it favorable for some processing [2].

The objectives of this study are to determine a suitable acidity regulator for yellow passion fruit drinking yoghurt according to national legislation, to determine the effect of an acidity regulator on physicochemical, microbiological and sensory acceptance during storage of yellow passion fruit drinking yoghurt and to enhance the sensory and physicochemical properties of passion fruit drinking yoghurt which may alter due to high acidity.

Materials and Methods

Passion fruit pulp was obtained from a third-party local supplier. Ultra-High Temperature (UHT) treated milk packets produced by a local dairy company were used to prepare drinking yoghurt. Food grade $Ca(OH)_2$ was used as the acidity regulator in the experiment and the chemical was purchased from MAS chemicals, Nugegoda, Sri Lanka.

Preparation of drinking yoghurt

UHT-treated milk was measured in to a stainless-steel container and then, potable water was added to the milk. The container was placed in a water bath and the mixture was heated up to 70 °C while stirring. When milk reached this temperature, 77.0 g of sugar and 1.25 g of gelatin were added and stirred well until dissolved. Then the heating was continued until the temperature rises to 95 °C and kept at that temperature for six minutes. The sample was cooled down to 44 °C and two tablespoons of yoghurt manufactured on the previous day were added as the starter culture. It is composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. After that, the drinking yoghurt mix was placed in the incubator at 44 °C. The pH of the mix was measured in 30 minutes. Once pH arrives at 5.0, the drinking yoghurt mixture was taken out of the incubator and placed in the refrigerator at 4 °C for 12 hours. After the refrigeration period, the curdle was broken and filtered using a 0.3 mm mesh size kitchen strainer. The filtered product was filled in a PET container and stored in the refrigerator at 4 °C.

Preparation of passion fruit pulp

The titratable acidity of passion fruit juice was measured using the AOAC method (AOAC, 2000). Based on the grams of citric acid, the amount of $Ca(OH)_2$ required to neutralize the citric acid in passion fruit was calculated by considering the assay of $Ca(OH)_2$. From the exact amount of $Ca(OH)_2$ required to neutralize citric acid in a particular amount of passion fruit juice, 63% was measured and added to the pulp. When the exact amount of $Ca(OH)_2$ required to neutralize citric acid was added to the pulp it altered the color and flavor. 63% from the exact amount of $Ca(OH)_2$ was added in order to retain the color and natural flavor as well as to maintain the pH of the passion fruit pulp at 4. This amount (63%) was selected by performing a series of trials. Then the mixture was covered with an aluminium foil and then with cling wrap and stored in the refrigerator at 4 °C until precipitating calcium citrate.

Incorporation of treated passion fruit juice into drinking yoghurt

7% of $Ca(OH)_2$ treated juice was gradually added to the drinking yoghurt based on the requirement of the company and mixed well. The beaker was covered by an aluminium foil and placed in the refrigerator at 4 ℃ for storage analyses. The control sample was prepared by adding 7% of untreated juice to drinking yoghurt and following the same procedure.

Determination of titratable acidity and pH

The acidity of the drinking yoghurt samples was determined by following AOAC method and pH was measured using Apera pH850 – DP portable pH meter for dairy products.

Determination of the moisture content of the samples
The moisture content of the drinking yoghurt samples was measured by KERN – DLB Electronic Moisture Analyzer.

Determination of fat content of the samples
Gerber method was followed to measure the fat percent in drinking yoghurt samples (AOAC, 2000).

Sensory Evaluation
The sensory attributes (color, aroma, taste, thickness, mouthfeel and overall acceptability) were evaluated by 30 untrained panelists from the University of Sri Jayewardenepura using a 5-point hedonic scale.

Statistical Analysis
Collected data were analyzed by One-way ANOVA to test the significance of variables (α=0.05) and by Tukey Test for comparison of data, using the Minitab 17 software package. Significance difference was determined using independent sample T-test and $p<0.05$ was considered significantly different in all experiments.

Results and Discussion

pH of the drinking yoghurt
According to the table 01, there is a significant difference between the pH of both types of drinking yoghurt samples indicating that the pH of the control sample is significantly lower than the $Ca(OH)_2$ added drinking yoghurt sample.

Table 01. pH, acidity, moisture and fat content of drinking yoghurt samples during 12 days of storage period at 4℃

Parameter	Sample	Day 01	Day 04	Day 08	Day 12
pH	Control	4.040.01^{b}	4.020.00^{b}	4.000.01^{b}	3.960.02^{b}
	$Ca(OH)_2$ added	4.620.01^{a}	4.580.00^{a}	4.560.00^{a}	4.520.00^{a}
Acidity (% in means of lactic acid)	Control	0.680.02^{bq}	0.710.01^{aq}	0.760.02^{ap}	0.770.01^{ap}
	$Ca(OH)_2$ added	0.460.01^{ar}	0.480.01^{br}	0.520.01^{bq}	0.540.01^{bp}
Moisture (%)	Control	79.870.03^{b}	79.250.02^{b}	78.910.03^{a}	78.800.02^{a}
	$Ca(OH)_2$ added	80.050.05^{a}	79.780.03^{a}	79.210.01^{b}	78.880.04^{a}
Fat (%)	Control	2.40.12^{a}	2.30.15^{a}	2.30.15^{a}	2.30.21^{a}
	$Ca(OH)_2$ added	2.50.27^{a}	2.50.15^{a}	2.50.06^{a}	2.40.15^{a}

Values are meanstandard deviation in triplicates. Values that do not share a letter (*a, b*) in the same column are significantly different between the samples. ($p<0.05$)

Values that do not share a letter (*p, q, r*) in the same row are significantly different between two samples on different days. ($p<0.05$)

The $Ca(OH)_2$ added sample has a higher pH compared to the control sample as $Ca(OH)_2$ acts as an acidity regulator and elevated the pH of passion fruit pulp. Food additives such as acidity regulators are added to different food products to develop an acidity that is favorable for certain food processes. However, according to table 1, the pH of both samples dropped over time due to the breakdown of the lactose sugar present in the milk into lactic acid as a result of the action of microbes in yoghurt culture. pH changes within the same type of yoghurt samples could be observed as a result of differences in ingredients used in production, differences in starter culture, incubation time-temperature combination and storage conditions.

Determination of the titratable acidity

The acidity of the control sample is significantly higher than the $Ca(OH)_2$ added drinking yoghurt sample. When acid-rich fruit pulp is added to drinking yoghurt, the acidity of the drinking yoghurt is elevated. According to findings of available studies, the drinking yoghurt prepared by incorporating mango pulp had a significantly high acidity compared to plain drinking yoghurt. In addition, the acidity of Annona fruit pulp added yoghurt had higher acidity than the plain yoghurt [3].

Determination of the moisture content of the samples

The moisture content of the control sample is significantly lower than the $Ca(OH)_2$ added drinking yoghurt sample on day 01, day 04 and day 08. But, the moisture content of the control sample is lower than the $Ca(OH)_2$ added yoghurt sample on day 12. The moisture content of both the drinking yoghurt samples has reduced gradually over the storage period. Research carried out by Hassan and Amjad to evaluate the nutritional value of yoghurt prepared by different starter cultures and to analyze the physicochemical properties during storage also revealed that the moisture of yoghurt has been reduced over time [4].

Determination of the fat content

Based on the table 01, there is no significant difference between the fat content of different types of drinking yoghurt samples. But, the fat content of the control sample is lower than the $Ca(OH)_2$ added yoghurt sample.

The fat content remained generally stable throughout storage, implying that there had been no effect over time. According to the specifications of SLS on drinking yoghurt, the minimum fat content should be 2.2% and both the drinking yoghurt samples are within the recommended level.

Sensory Analysis

$Ca(OH)_2$ added yoghurt sample has a significantly better mouthfeel than the control sample**.** But, overall acceptability, aroma, thickness and taste are not significant at a 0.05 significant level. $Ca(OH)_2$ added yoghurt sample is significantly better than the control sample since most of the sensory characteristics relevant to the $Ca(OH)_2$ added yoghurt sample is better than the control sample.

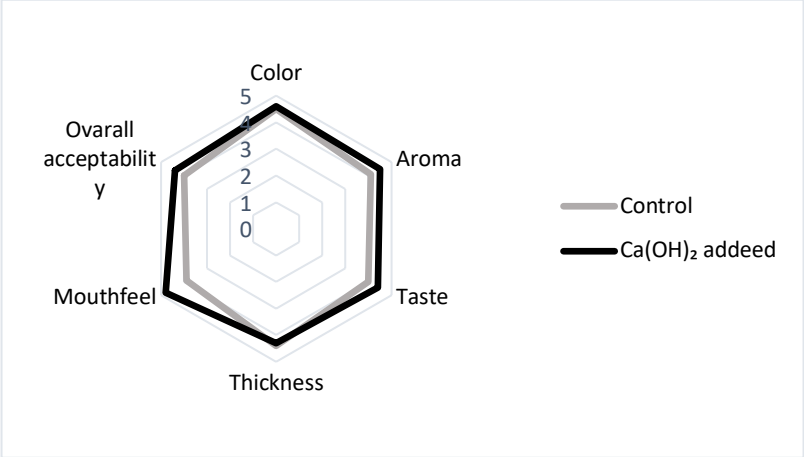

Figure 1. Sensory evaluation results for prepared drinking yoghurt samples

Yeast and mold count

According to the independent sample T-test, there is not enough evidence to reject the null hypothesis (p>0.05). So, there is not a significant difference between the yeast and mold count of the two types of drinking yoghurts.

Table 02. Yeast and mold count in drinking yoghurt samples during 12 days of storage period at 4 °C

Sample	Yeast and mold (log CFU/mL)		
	Day 01	Day 07	Day 14
Control	$2.0380.27^{ar}$	$2.8270.06^{aq}$	$3.8250.06^{ap}$
$Ca(OH)_2$ added	$1.9520.07^{ar}$	$2.8640.07^{aq}$	$3.8210.01^{ap}$

Values are meanstandard deviation in duplicates. Values that do not share a letter (*a*) in the same column are significantly different between the samples. (p<0.05)

Values that do not share a letter (*p, q, r*) in the same row are significantly different between two samples on different days. (p<0.05)

The yeast and mold count of both the drinking yoghurt samples was within the SLS standards till day 07 [5]. In between day 07 and day 14, the recommended limit has exceeded making the product unfavorable for human consumption. The shelf life of a selected commercially available drinking yoghurt which excludes

preservatives is 11 days. Therefore, the storage period of $Ca(OH)_2$ added drinking yoghurt sample haven't extended with the addition of the acidity regulator.

Conclusions and Recommendations

Obtained data on pH and acidity values in this study illustrates that $Ca(OH)_2$ can be used to reduce the acidity level in passion fruit without causing any negative impact on the physicochemical and sensory attributes of the passion fruit incorporated drinking yoghurt. Through a series of trials, it was identified that 63% from the exact amount of $Ca(OH)_2$ required to neutralize citric acid in passion fruit juice is suitable to be added to the pulp.

Dairy products including yoghurts, are rich sources of calcium yet still several people suffer from non-communicable diseases such as osteoporosis due to dietary calcium deficiency. Therefore, future work can be focused on examining whether $Ca(OH)_2$ acts as a calcium supplement in drinking yoghurt.

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EXTRACTION OF MICROCRYSTALLINE CELLULOSE FROM PEANUT SHELL BY CHEMICAL TREATMENT AND ITS FTIR ANALYSIS

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Introduction

Groundnut shells are by-product of the extraction of groundnut seeds from its pod, where it is an abundant agro-industrial waste product that degrades extremely slowly in the natural environment. Groundnut shells on the other hand contain a variety of bioactive and functional compounds that are helpful to humans. Commercially, it is utilized as a feedstock, food, fertilizer filler and carrier for bio-filters. However, need for greater environmental sustainability by reducing municipal ver, the majority of deserted groundnut shells are burned or buried, polluting the environment.

Microcrystalline cellulose is a cellulose derivative derived from a natural fibre that is suitable for medicinal, cosmetic, food and plastic composite applications due to its compatibility, hydrophilicity, acid insolubility and biodegradability. Fibrous wood pulp is the most prevalent source of microcrystalline cellulose. Heat and pressure are used to hydrolyze the pulp. The cellulose polymer breaks down in the presence of water and acid during hydrolysis. Small chain polymers or micro crystals are formed from cellulose polymers in the pulp. During the acid hydrolysis process, the acid solution penetrates cavities in the amorphous zone before the crystalline region and breaks down the cellulose glycosidic link, resulting in a micro-scale crystalline structure of small size. The amorphous component of cellulose is mostly converted to glucose during acid hydrolysis.

Peanut shells include lignin microfibrils, cellulose and hemicelluloses which are organized into microfibrils just like other cellulosic materials. The chemical compositions of peanut shell fibre were determined to be 35.7% cellulose, 18.7% hemicellulose, 30.2% lignin and 5.9% ash. As a result, using peanut husk (shell) as a natural filler in polyolefins will open up a new path for converting agro-waste into usable resources for the plastics sector. This contributes to the solid waste and generating “waste to riches”.

Materials and methods

*Peanut shell collection and preparation*Peanut shells were collected from the Murikandy area, Mullaithivu. Shells were cleaned and washed with tap water several times to remove dirt and debris. The method described by Punnadiyil et *al*., 2016 was used with slight modification. Washed peanut shells were allowed

to dry at 60°C for 18 hours using a cabinet dryer. Dried shells were ground into 90 µm using the grinder. Peanut shell powder was stored in an airtight container for further analysis.

Extraction of microcrystalline cellulose from peanut shell.

The 50 g of peanut shell powder was weighed. Peanut shell powder was added into a conical flask. The 500 mL of 1.5% NaOH was added to that conical flask. The solution with peanut shells was heated at 75°C for 1 hour in the water bath. The treated solution was vacuum filtered until the filtered solution became a clear solution. It was dried at 60°C for 24 hours in an oven. The end product is called an "Alkali treated sample" [2]. The 1 g of Alkali treated sample was refluxed with 25 mL of HNO_3:Ethanol mixture (1:4) ratio. It was carried out at boiling temperature for one hour. This process is called "Refluxation". Finally, it was washed with cold distilled water and filtered through the vacuum pump. The final product was dried at 90°C for one hour with slight modification. The end product was "Cellulose" [3]. The 50 g of bleached cellulose was poured into 2.5 N 1.2 L of HCl at boiling temperature for 15 minutes. Then this hot-acid mixture was poured into cold water and mixed well and stand for overnight. Then it was washed and dried at 60°C for 60 minutes [4]. The end product was microcrystalline cellulose.

Sample preparation for FTIR

Peanut shell powder, cellulose powder and microcrystalline cellulose were used for the analysis. Each sample was mixed well by crushing with KBr (FT-IRgrade, ≥99% trace metals basis, Sigma Aldrich) and pellets were made by pressing in dye.

FTIR condition

FTIR spectra of powder samples were recorded using a Fourier transform Infrared Nicolet spectrophotometer coupled with ATR (Attenuated Total Reflectance) mode with zinc selenate crystal. FTIR spectra were collected in mid-infrared region of 4000 – 500 cm^{-1} by co-adding at 32 scans, resolution of 4 cm^{-1} for samples. A background spectrum of pure KBr pellet was used to correlate all spectra. Each scan was followed by obtaining a new reference background spectrum. For FTIR analysis, a small number of powdered samples were used. FTIR spectra were interpreted using Microsoft Excel Software.

Results and discussion

The infrared spectrum is divided into three wavenumber regions: Far-IR (<400 cm^{-1}), mid-IR (400-4000 cm^{-1}) and near-IR (4000-13000 cm^{-1}). The mid-IR spectrum is the most commonly used in sample analysis. The mid-IR (Infrared) spectrum can be divided into four regions:

I. Double bond region (1500-2000 cm^{-1})
II. Triple bond region (2000-2500 cm^{-1})
III. Single bond region (2500-4000 cm^{-1})
IV. Fingerprint region (600-1500 cm^{-1}) [5].

Modifications in the intensity of FTIR bands indicated that changes had occurred during chemical treatments. Table 1 provides the details of the different compounds in different wavelengths of the peanut shell powder sample.

I. Single bond region (2500-4000 cm^{-1})

There are more than five peaks, indicating that the molecule being tested is not a simple compound [5]. The spectra show that the absorption band arising at around 3600 – 3100 cm^{-1}, informing there is a hydrogen bond in the material as well as had significant contour where the peak was broad and blunt. This is generally attributed to stretching vibration of O-H groups associated with each sample. In this structure, no peak levels at 3200 – 3000 cm^{-1} were found which indicates the aromatic structure. A stretching was observed in the 2923 – 2930 cm^{-1} region which is responsible for the C-H bond.

II. Triple bond region (2000 – 2500 cm^{-1})

There is a C≡C absorption band that peaks at 2200 cm^{-1} in the PSP sample.

III. Double bond region (1500 - 2000 cm^{-1})

Pure PSP exhibited high absorption frequencies of 1735 cm^{-1} and 1247 cm^{-1} respectively, corresponding to C=O stretching of acetyl and uronic esters groups from lignin and hemicelluloses and C-O out-of-plane stretching vibrations of phenyl groups in lignin. During the chemical treatment, these two bands progressively faded. The lack of these two bands in microcrystalline cellulose suggests that lignin and hemicelluloses have been completely removed (Viera *et al*., 2007). The carbonyl group's stretching and bending vibration peaks were located at 1748 cm^{-1} and 1243 cm^{-1}. The reduction of carbonyl group intensity showed that hemicellulose was removed. The signal at 1639 cm^{-1} increased, indicating an increase in hemiacetal and carboxyl groups as well as an increase in oxidized groups, indicating that the percentage of oxidized cellulose increased [3]. The peaks at 1424 cm^{-1} and 1510 cm^{-1} correspond to cellulose's scissoring and O-H bending vibrations, respectively.

Thus, FTIR analysis confirms the presence of cellulose and the significant removal of hemicellulose and lignin in microcrystalline cellulose.

Conclusion

Physical and chemical changes of the micro-crystalline cellulose and cellulose obtained by the reaction from the peanut shell were analyzed by fourier transform infrared spectroscopy. Fourier transform infrared spectra (FTIR) confirmed the removal of hemicelluloses and lignin. Thus, it proves the isolation of cellulose and microcrystalline cellulose from peanut shell powder during the different stages of chemical treatment.

Table 1 Characteristics spectra bands of extracted powder

Assignment	Wavenumber (cm^{-1})	
	Experiment	Literature
C-O stretching of the ester group	1223	-1247
O-H bending vibration	1445	-1424
O-H bending vibration	1513	-1510
Hemiacetal and carboxyl groups	1648	-1639
C=O stretching of the carbonyl group	1748	1745-1750
C≡C absorption	2217	-2200
Stretching of C-H bond	2924	2923-2930
O-H stretching	3345	3100-3600

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgement

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OPTIMIZING BIOSYNTHESIS OF FEO NANOPARTICLES USING *Marsilea* q*uadrifolia* (L.) LEAF EXTRACT

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Introduction

Nanotechnology is widely applicable worldwide due to its advanced features. Nanoparticles (NPs) are preferred in many applications because of their large surface area. Maghemite or magnetite has unique properties of iron oxide (FeO) nanoparticles smaller than 20 nm [1]. Unique properties such as super magnetism are of interest in the extraction of Fe nanoparticles. Therefore, FeO NPs are used for various application fields in science and technology. Literature is available on synthesized FeO NPs using different plant parts such as stem, roots, and leaves. In the bottom-up approach, a number of methods have been developed to synthesize FeO Nanoparticles such as chemical, physical and biological methods. However, chemical and physical methods including sol-gel process, chemical co-precipitation, and chemical methods such as vapour deposition are highly expensive and are being used as stabilizing agents like Sodium Dodecyl Sulphate, which produces very hazardous by-products [2]. Because of these problems, researchers are finding an environmentally friendly and sustainable method for nano manufacturing. Biosynthesis is the best solution using biological materials for NPs synthesis [3]. It is claimed to be an environmentally friendly and more sustainable method than chemical and physical methods. Plant bacteria, fungi, yeast, etc. used for the extraction of bio-based NPs. Plants or microorganisms contain bioactive compounds, and enzymes act as reducing and capping agents. Literature provides evidence that biosynthesis methods show advantages over conventional methods. Several reports are available on FeO NPs synthesis through green mode. Reported that synthesis of ferrous oxide NPs using *Hibiscus rosa-sinensis,* the synthesis of iron nanoparticles using an aqueous extract of *Musa* and further investigated the resistances against the pathogenic bacteria [4]. Synthesis of iron nanoparticles using an aqueous extract of *Musa Ornate* is reported [5].

In the present study, iron Oxide nanoparticles are synthesized using *Marsilea* q*uadrifolia* leaf extract, used as both capping and reducing agent and the optimum salt concentration, and *M.* q*uadrifolia* leaf extract to optimize the production.

Materials and methods

Plant extract preparation

M. q*uadrifolia* plant was collected from the paddy field at Balangoda, Sri Lanka in July 2022 and surface sterilized with running tap water for 30 minutes to remove debris and other contaminations, followed with deionized water and dried in air direr at 42°C overnight. About 5 gm of ground leaf powder was kept in a beaker containing 80 ml water and boiled for 45 min. The extract was cooled and filtered with Whatman filter paper no.1 three times and the extract was stored at 4°C for further use.

FeO nanoparticle synthesis

FeO NPs were prepared by adding 1 M (100 ml water) of Ferrous Nitrate. 5 mL of plant extract was added to three separate test tubes followed by adding ferrous Nitrate 5 ml and 10 ml in the ratio of 1:1, 1:2, 2:1 (plant extract: salt) respectively. The solution was maintained at 80°C for an hour and then reactions were allowed to progress at room temperature. During this time, the colour of the extract reaction changed from translucent pale to black and light green to black, indicating the formation of FeO NPs. Observations were made at 24 hrs. Each reaction mixture was centrifuged at 14000 rpm for 20 min, the supernatant was discarded, and the remaining pellet was washed one time with de-ironized water.

Characterization of synthesised Nanoparticles

UV-Vis spectroscopy and SEM analysis

UV–Vis spectrometry analysis was carried out using CT-2600 UV-Vis Spectrometers (BioTek©) with a resolution of 1 nm between 200 and 700 nm. The resulting FeO NPs pellets were re-suspended in deionized water and used for characterization. SU6600 Scanning Electron Microscope (HITACHI) was used for the morphology, size, and analysis of the particle distribution of iron oxide nanoparticles, and microscopic structure was observed at 10.00 KV under multiple (KX) magnifications.

Results and Discussion

The preliminary test to observe the colour change confirmed the formation of Fe NPs. Colour of the plant extracts was turn to darker. Comparative to the 2:1 concentration, 1:1 and 1:2 concentration colours were darker. FeO NPs formation was confirmed by the deviation of pH value of the reaction mixture. It was changed as given in Table 1. The pH value reduced drastically with the formation of nanoparticles. According to [1], pH drop was observed with mango extraction from 5.12 to 2.16, with clove from 4.22 to 1.88, with green tea from 5.37 to 2.65.

Table 1. Variation of the pH value of the leaf extracts, Ferrous Nitrate solution, and the reaction mixtures used for the study

Sample	pH
Marsilea quadrifolia (L.). leaf extract	5.10
Ferrous Nitrate	0.55
Reaction mixtures	
1:1	0.56
1:2	0.45
2:1	0.23

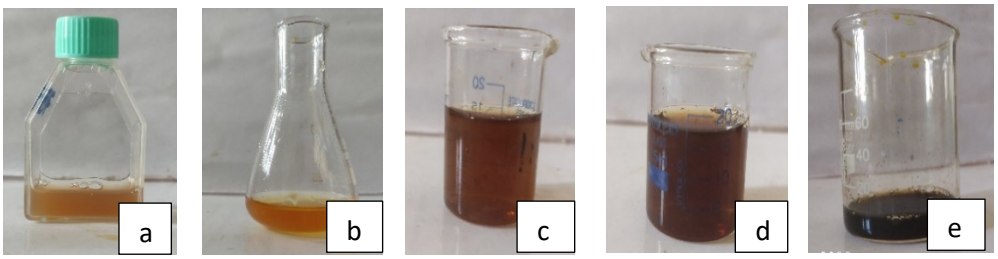

Figure 1. Phases to transform the mixture to FeO NPs *via* bio synthesis. (a); *M. quadrifolia l*eaf extract, (b); colour of Ferrous Nitrate (c); 2:1 leaf extract: salt (After 24 hrs), (d); 1:1 leaf extract: salt(After 24 hrs), (e); 1:2 leaf extract: salt (After 24 hrs).

UV-Vis Spectrum analysis

Figure 2 shows UV-Visible spectrum of *M. quadrifolia* leaf extract. Figure 3 shows the UV- Vis spectrum of the *M. quadrifolia* FeO NPs characteristic absorption peak at the 280-290 nm wavelength.

According to [2] the characteristic absorption peak formation of NPs, nanoparticles synthesized using *Pometia pinnata* was observed at 272-297 nm. Synthesis of NPs using *Aesculus hippocastanum*, which UV-Vis spectroscopy was observed in the wavelength range of 250-300nm [4].

SEM Analysis

Shape and average particle sizes of FeO NPs synthesis using *M. quadrifolia* were determined by using the SEM image analysis. Figure 5 shows SEM images from ferric oxide nanoparticles, it shows the structural features of FeO nanoparticles and Figure 4 shows the average practical sizes distribution of the synthesized FeO NPs. Synthesized NPs are cubic shaped and have approximately average particle size distributed around 30- 39 nm. Results were consistent with previous studies. Observed 27.91 to 40.94 nm size particles were observed Iron oxide nanoparticle synthesis using trigonella and tomato extracts [5]. Further, according to [4] size and the shape of the particle generally depended on the concentration and nature of the plant extract.

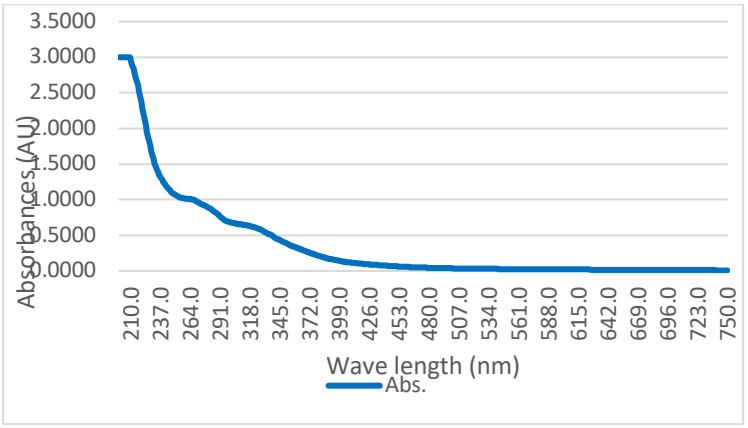

Figure 2. UV-Visible spectroscopy of *Marsilea quadrifolia* (L.). leaf extract

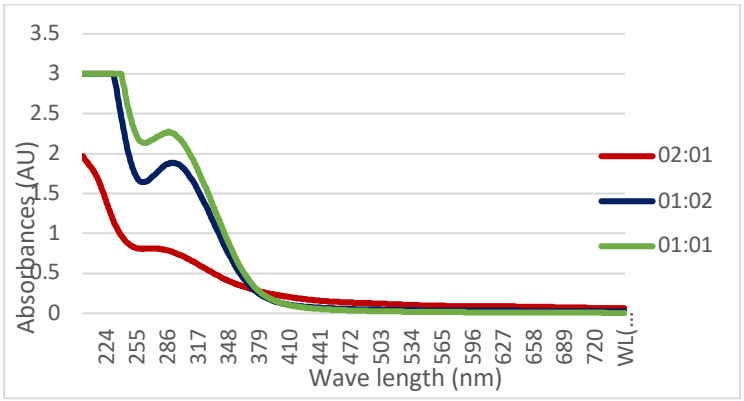

Figure 3. UV-Visible spectra image showing absorption peaks.

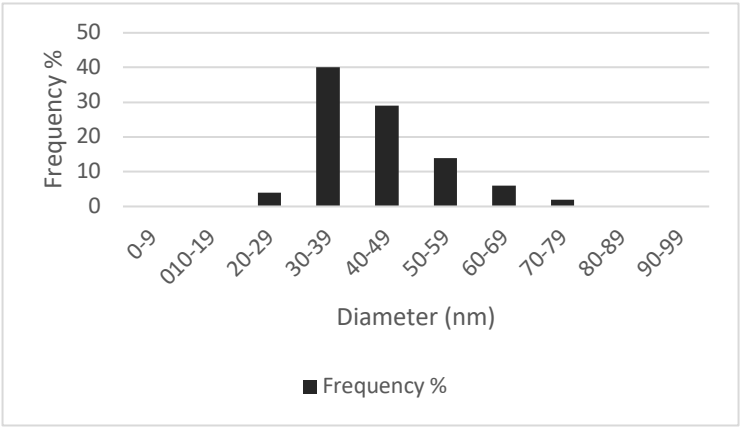

Figure 4. Frequency of particle size distribution of FeO NPs

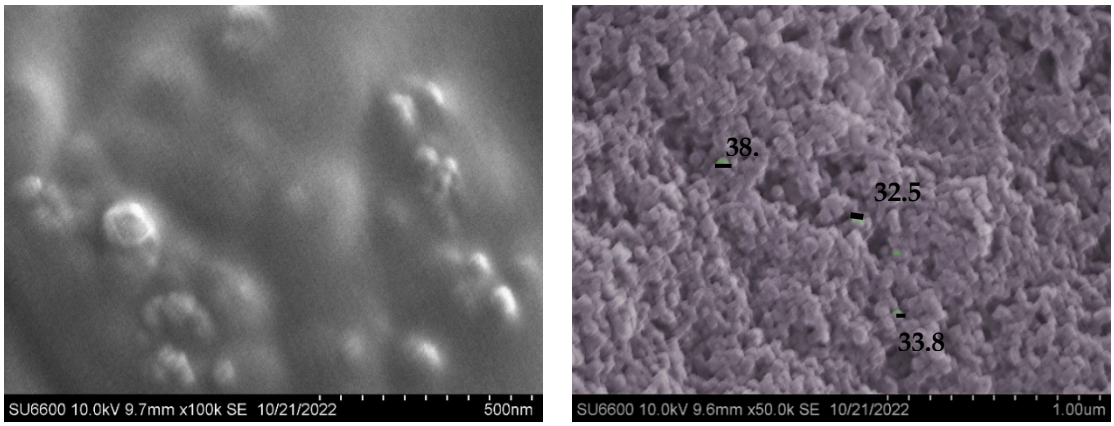

Figure 5. SEM images from Ferric Oxide nanoparticles

Natural iron oxide exists in the number of different **w**ays; magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), and hematite (α-Fe_2O_3). According to their phase, chemical composition and properties also differ from each other [1]. Moreover, in the present study the phase colour changed as; iron oxide magnetite has a black colour, maghemite has light brown colour, and hematite has red colours.

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Conclusion

The present study successfully synthesised FeO NPs using the leaf extract of *M. quadrifolia*. Synthesized FeO NPs was characterized by various techniques. First it was confirmed through the preliminary test and then through Uv-Visible spectrum and SEM imaging, Green production of FeO NPs will be a promising area in Nanotechnology. As biosynthesis lacks the usage of toxic chemicals and excessive energy consumption, it could be an eco-friendly sustainable method. Physical and chemical methods have shown several drawbacks. Therefore, it is possible to conclude that potential of *Marsilea quadrifolia* leaf extract to act as a stabilizing agent and the best concentration ratio of salt and *M. quadrifolia* leaf extract is 1:1 to optimize the synthesis of FeO nanoparticles.

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Fatty acid profile and Heavy metal composition of some Hibiscus Flowers of Sri Lanka

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Introduction

Hibiscus spp. is an ornamental plant that belongs to the family Malvaceae that has so many therapeutic properties due to the presence of various active biochemical compounds. The genus *Hibiscus* is with more than 300 species, that are widely distributed in temperate, tropical, and sub-tropical regions in the world especially in the Asian subcontinent. The nutritional and toxicological composition of Hibiscus flowers varies due to the variety of the flower, genetic information, environmental condition, of the plant. The flowers of *Hibiscus sp.* rich in fatty acids, phenolic compounds, flavonoids, carbohydrates, proteins, etc [1].

Generally, Hibiscus flowers are used in cosmetics, medicine, food, and beverage production in some countries. But in Sri Lanka, flowers of Hibiscus are commonly used as a medicine in Ayurveda and in cosmetic industries but not popular in food and beverage industries. The flower Hibiscus is beneficial to human health due to the presence of antioxidants, phenolic compounds, vitamin C and minerals. Especially the red flower is used against paralysis and to control menstruation. Furthermore, the extracts in the flowers act against bronchitis, gonorrhea, fever and coughs [2]. Besides, Hibiscus is used as a key ingredient in cosmetics because the flowers have anti-ageing, anti-acne properties due to the presence of antioxidants, free radical scavenging activity and antimicrobial activity [3], [4]. The flowers of *Hibiscus spp.* are observed to be promoters of hair growth and have anti-greying properties [5].

Hibiscus spp. characterized as an alternative source of edible oil because the extract of the flower is rich source of unsaturated fatty acids such as oleic, linoleic. The flower calyxes contain omega-3 fatty acids which play an important role in human physiology [6]. Besides due to the environmental and atmospheric pollution, the Hibiscus flowers consist of heavy metals such as As, Cd, Pb and Hg which can cause acute and chronic toxic effects for human beings.

The objectives of this investigation were to assess the fatty acids composition and heavy metal content in *Hibiscus spp.* for the human consumption.

Materials and Methods

The eleven varieties of flowers of *Hibiscus spp.* were collected from Anuradhapura area. In this study, the collected samples were labelled as A to K.

From those eleven samples, all belongs to single species, *Hibiscus rosa sinensis* except MR-J. MR-J belongs to the species, *Hibiscus schizopetalus.*

Fatty acid profile of Hibiscus spp.

The fresh flower petals of Hibiscus flowers were oven dried at $400C$. Then the fat content in Hibiscus flowers were extracted by Soxhlet extraction method. The extraction process was carried out for about 6 hours using 100.00 mL petroleum ether and then the extracted solution was concentrated using rotary evaporator at $400C$ to remove petroleum ether. Then 50.00 mg of extracted fat samples in eleven samples were put into screw capped tubes separately. Then 1.00 mL of 0.5 M methanol/NaOH was added into each sample. The screw cap was tied and boiled for about 5 minutes until the crude sample was dissolved completely in the solution. The solution was cooled to room temperature. 1.50 mL of CH_3OH/BF_3 and 1.00 mL of 0.1% hydroquinone were added to each sample. The cap was tied again and kept in a hot water bath for 5 minutes. The solution was kept aside for cooling and 2.50 ml of normal heptane was added to each sample and vortexed for 1 minute. The tube was kept aside until the phases were separated completely. The organic layer was transferred into vials. Finally, the fatty acids in the samples were analyzed by Gas Chromatography - Mass Spectrometry (GC-MS, Shimadzu QP 2020)[7]. The peaks obtained were identified by using standards and mass library available in the GC-MS.

Heavy metal content in Hibiscus spp.

The heavy metal content in Hibiscus flowers were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after preparation of samples by microwave digestion. Dried and grounded Hibiscus samples (0.25g each) were accurately weighed and placed in a Teflon digestion vessel. 5.00 ml of conc. HNO_3 and 1.00 ml of H_2O_2 were added to each sample and then digested using microwave digestor (Milestone, ETHOS EASY) acid digestion. The samples were initially kept at $2000C$ for 20 minutes and again at $2000C$ for 15 minutes in the power of 1800 W. The digested samples were cooled to room temperature. The digested samples were diluted up to 50.00 mL with DI water and then the As, Pb, Cd and Hg contents in each sample were analyzed by using the ICP-MS (Perkin Elmer NexIon 2000) [8].

Results and Discussion

Analysis of Fatty Acid Methyl Ester (FAME) was determined to provide the quality and availability of diverse fatty acids and quantify the fatty acids in the Hibiscus flowers.

Table1. Fatty acid composition in the flowers of *Hibiscus spp*.

Fatty acid	A	B	C	D	E	F	G	H	I	J	K
Octanoic acid (C8:0)	0.38	0.37								0.72	
Decanoic acid (C10:0)	2.70	1.62		2.00	4.38	6.08	6.29	4.55	3.69	3.22	3.13
Dodecanoic acid (C12:0)	2.49	6.48	3.57	6.74	6.07	10.1	7.52	7.98	6.76	8.01	9.36
Tetradecanoic acid (C14:0)	6.73	4.92	4.48	3.19	7.68	7.79	10.2	5.77	5.85	5.63	6.32
Pentadecanoic acid (C15:0)		0.26									
Hexadecanoic acid (C16:0)	25.58	27.52	26.60	28.98	15.51	26.03	25.40	27.78	24.26	26.35	31.63
Heptadecanoic acid (C17:0)	0.72	0.60				8.22			0.56		
Stearic acid (C18:0)	7.54	10.17	8.28	9.24	9.08		6.29	8.15	7.23	9.61	7.90
9-Octadecenoic acid (E) (C18:1)	9.50	7.09	5.99	8.32	4.18	4.84	6.62	5.10	3.71	3.91	4.32
9,12-Octadecadienoic acid (Z, Z) (C18:2)	34.11	27.29	40.60	37.33	21.98	25.10	22.21	21.76	30.93	27.10	23.52
Eicosanoic acid (C20:0)	1.25			1.49	1.62		2.52	2.09	1.22	1.47	1.94
9,12,15-Octadecatrienoic acid (Z, Z, Z) (C18:3)	6.84	3.89	5.55		22.36	4.69	8.81	9.37	7.26	12.52	4.11
Cis-11-Eicosenoic acid (C20:1)				1.28							
Docosanoic acid (C22:0)	2.17	3.31	2.35	1.43	1.49	3.01	2.01	7.45	3.64	1.47	2.92
Tetracosanoic acid (C24:0)		4.65	1.30		5.63	4.13	2.14		4.88		4.83

The most abundant fatty acids in Hibiscus flowers are Hexadecanoic acid (Sample code: K), 9, 12-Octadecadienoic acid (A) and 9, 12, 15-Octadecatrienoic acid (E).

Table 2. Summary for fatty acid composition in the flowers of *Hibiscus spp.*

Sample ID	Fatty acid **(%)**			
	Total saturated fatty acids	**Total monounsaturated fatty acids**	**Total polyunsaturated fatty acids**	**Total Omega-3**
A	49.56	9.50	40.95	6.84
B	59.90	7.09	31.18	3.89
C	46.58	5.99	46.15	5.55
D	53.07	9.60	37.33	7.18
E	51.46	4.18	44.34	22.36
F	65.37	4.84	29.79	4.69
G	62.37	6.62	31.02	8.81
H	63.77	5.10	31.13	9.37
I	58.09	3.71	38.19	7.26
J	56.48	3.91	39.62	12.52
K	68.03	4.32	27.63	4.11

The total saturated fatty acid percentage determined for Hibiscus flowers were ranged from 46.58% (C) to 68.03% (K) while the total monosaturated fatty acid percentage was ranged from 3.71% (I) to 9.60% (D). Among the Hibiscus varieties studied, sample: C (46.15%) showed the highest percentage of total polysaturated fatty acid while sample: K (27.63%) has the lowest percentage of total polysaturated fatty acid. A higher percentage of omega was observed in the sample: E (22.36%).

The contents of heavy metals As, Pb, Cd and Hg in Hibiscus flowers are given below.

Table 3. Heavy metal content in flowers of *Hibiscus spp.*

Sample ID	As	Pb	Cd	Hg
	Average Concentration (μgkg^{-1})	Average Concentration (μgkg^{-1})	Average Concentration (μgkg^{-1})	Average Concentration (μgkg^{-1})
A	12.117 ± 0.28	147.401 ± 1.44	17.028 ± 0.62	18.462 ± 0.99
B	14.218 ± 0.24	177.640 ± 1.94	31.084 ± 1.18	18.861 ± 0.36
C	19.003 ± 1.21	464.015 ± 4.61	31.605 ± 0.27	12.090 ± 0.60
D	14.218 ± 0.41	469.562 ± 7.22	23.894 ± 0.75	20.282 ± 0.47
E	18.920 ± 0.14	543.155 ± 3.04	26.507 ± 0.98	13.314 ± 0.65
F	27.476 ± 0.23	229.492 ± 3.69	69.718 ± 1.12	9.198 ± 0.48
G	18.274 ± 0.24	157.084 ± 5.08	11.162 ± 0.37	17.425 ± 0.84
H	16.435 ± 0.21	767.731 ± 18.10	15.555 ± 0.55	7.551 ± 0.38
I	19.804 ± 0.45	151.265 ± 1.42	17.737 ± 1.06	9.241 ± 0.45
J	12.634 ± 0.25	141.486 ± 1.86	37.989 ± 0.57	43.159 ± 0.49
K	19.833 ± 0.07	109.626 ± 3.89	20.955 ± 0.50	4.232 ± 0.14

Data represented as mean ± SE (n=3).

The findings of this study, exhibit heavy metal concentration varies with the variety of Hibiscus flower. The Pb concentration was ranged from 109.63 ± 3.89 μgkg^{-1} (K) to 767.73 ± 18.10 μgkg^{-1} (H), showing a higher concentration than the maximum permissible level in some flower types. The highest and lowest As concentrations were reported from sample: F (27.48 ± 0.23 μgkg^{-1}) and sample: A (12.12 ± 0.28 μgkg^{-1}) respectively. As well as the maximum and minimum amount of Cd were reported from sample: F (69.72 ± 1.12 μgkg^{-1}) and sample: G (11.16 ± 0.37 μgkg^{-1}). The Hg concentration determined for Hibiscus flowers were ranged from 4.23 ± 0.14 μgkg^{-1} (K) to 43.16 ± 0.49 μgkg^{-1} (J).

Conclusions and Recommendations

Hibiscus flowers are rich in both saturated and polyunsaturated fatty acids. The highest amount of omega - 3 fatty acids were presents in sample E, showing considerable health benefits. The levels of As, Cd were within the acceptable level, but Pb concentration was higher than the maximum permissible levels of WHO/FAO in few samples, may indicate health risk.

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FOCUS AREA

Health

ACCEPTANCE AND PERCEPTION OF COVID-19 VACCINATION AMONG ADULTS IN THE GALLE DISTRICT

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Introduction

The COVID-19 (Corona Virus Disease-19) outbreak is reported in more than 220 nations and is regarded as the fifth global pandemic in history. The causative virus of COVID-19, which causes fatal pneumonia, is SARS-CoV-2 [1]. The first COVID-19 case reported in Sri Lanka in late January 2020 was a Chinese woman. On 2nd March 2020, the first local COVID-19 case was made public. As of 20th October 2022, there were 670,952 recorded cases and 16,772 fatalities in Sri Lanka, according to the WHO weekly epidemiological update [2]. During 2020-2022, Sri Lanka was affected by three COVID-19 pandemic waves.

Vaccines were suggested as the method of choice to prevent SARS-CoV-2 because there was no any other available method for an effective cure. Vaccines are typically used to manage various viral and parasitic infections to prevent millions of deaths annually. Given the significance of vaccination, scientists were striving for inventing vaccines by many methods [3]. Some research teams were able to successfully develop vaccines against SARS-CoV-2. The COVID-19 vaccination program in Sri Lanka started on 28th January 2021. As mentioned in the 'COVID-19 vaccination summary', Covishield, Sinopharm, Sputnik-V, Moderna and Pfizer vaccines were used to build up the immunity of the Sri Lankan population against the COVID-19 virus [2].

Initially, the public exhibit a big reluctance to COVID vaccines due to the lack of the awareness on COVID-19 vaccines. Present study was conducted to assess the acceptability and the reasons for refusing COVID-19 vaccines of the working-age population in the Galle district. The perception regarding the COVID-19 vaccines among the working-age population in the Galle district was also evaluated.

Materials and Methods

Sampling

A cross-sectional study was conducted in the Galle district. The purposively selected working-age (30 - 59 years) volunteers from 20 government and private sector institutes (four institutions from each divisional secretariat) by the simple random technique. The selected five divisional secretariats in the Galle district, were visited the institutions and the employers were educated on the study in

groups. And the employers who volunteered to participate were included in the study. This was done until the required sample size was achieved.

The required sample size was calculated using the formula suggested by Lwanga and Lemeshow [4] for finite populations.

$$n = \frac{z^2 pqN}{e^2 (N-1) + z^2 pq}$$

n = required sample size
z = Critical value of specified confidence = 1.96
p = Probable estimate of proportions of the characteristic of interest according to similar survey studies
q = 1-p
N = Size of population (Galle district population in 30 - 59 years range = 404,679) [5]
e = accepted amount of absolute error = 5%

$$n = \frac{1.96^2 \times 0.50 \times 0.50 \times 404{,}679}{0.05^2 (404{,}679-1) + 1.96^2 \times 0.5 \times 0.5} \times \frac{15}{100} = 441$$

The sample size was 441.

Ethical consideration
Ethical approval for this study was obtained from the Ethics Review Committee of the Faculty of Allied Health Sciences, University of Ruhuna, Galle (Reference No:.2021:07.15). Written informed consent was obtained from all participants.

Data collection instruments
A pre-tested, self-administered questionnaire was used to collect data from participants. Questionnaire sought data on socio-demographic characteristics of the participants, their perception on COVID-19 vaccines, details about the acceptance or rejection of the vaccine and reasons for refusing the COVID-19 vaccines. The socio-demographic characteristics included sex, age, and highest educational qualification.

Statistical analysis
IBM Statistical Package for Social Sciences (SPSS) software version 25.0 was used for the descriptive data analysis.

Results and Discussion
The majority of the sample comprised of females (n=307, 69.6%) and the mean age (±SD) of the participants was 40 (±8.1) years. The majority of participants (n=234, 53.1%) were at the age group of 30-39 years. Table 1 indicates the demographic characteristics of the participants.
Of the sample 94.8% (n=418) of participants had been fully or partially vaccinated (Sinopharm, AstraZeneca, or Pfizer) against COVID-19.

Table1. Demographic characteristics of participants

		No. of participants of the study	Percentage of participants of the study
Gender	Male participants	134	30.4%
	Female participants	307	69.6%
Education level	Without O/L	15	3.4%
	O/L	122	27.7%
	A/L	201	45.6%
	Degree	97	22.0%
	MA/MSc	6	1.4%
	MPhil/PhD	0	0.0%
Age (years)	Category 1 (30 – 39)	234	53.1%
	Category 2 (40 – 49)	122	27.7%
	Category 3 (50 – 59)	85	19.3%
Vaccination	Vaccinated (fully / partially)	418	94.8%
	Unvaccinated	23	5.2%

Out of the participants, 5.2% (n=23) had refused to obtain COVID-19 vaccines due to various reasons (Figure 1). Most of the participants (52%, n=12) had refused the vaccination due to a lack of trust in the safety of COVID-19 vaccines.

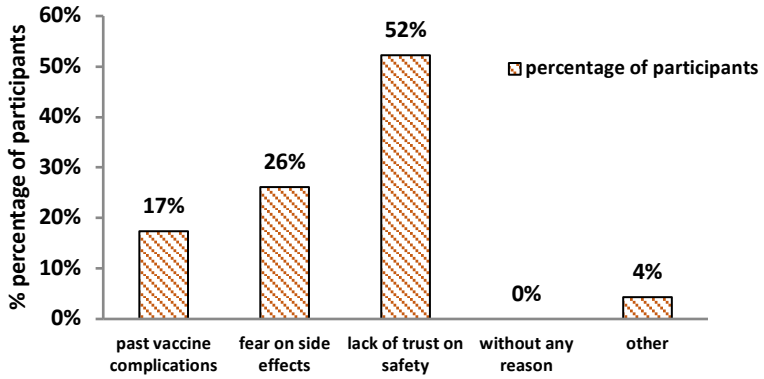

Figure1. Reasons for refusing COVID-19 vaccines of adults in the Galle District

Details about the participant's awareness on COVID-19 vaccines are indicated in the Table 2.

Table 2. Participant's awareness on COVID-19 vaccines

		Awareness of COVID-19 vaccines	Percentage of participants				
			Strongly agree	Agree	No idea	Disagree	Strongly disagree
Importance of COVID-19 vaccines	1.1	Vaccination will protect us from COVID-19	**24.7%**	**56.7%**	11.1%	5.4%	2.0%
	1.2	Vaccination will help to eliminate the disease	**14.3%**	**56.2%**	14.7%	11.3%	3.4%
	1.3	Vaccination will ensure the acquired immunity	**12.7%**	**67.1%**	14.3%	4.5%	1.4%
	1.4	It reduces the spreading of the disease	**12.7%**	**59.6%**	15.2%	11.6%	0.9%
	1.5	Life-long protection by the vaccination is not confirmed	**15.4%**	**55.6%**	20.0%	6.8%	2.3%
	1.6	COVID-19 vaccines are safe to be obtained	**12.0%**	**53.3%**	23.8%	7.9%	2.3%
Eligibility of COVID-19 vaccines	2.1	Neonates and immune-compromised persons should not be vaccinated	**6.6%**	**43.1%**	28.1%	17.9%	4.3%
	2.2	Vaccination is available for Sri Lankan pregnant mothers from 1st trimester	**6.8%**	**50.6%**	30.8%	8.4%	3.4%
	2.3	The vaccine could be taken by a person who recovered from the COVID-19	**7.3%**	**53.3%**	27.2%	10.4%	1.8%
Dosages of COVID-19	3.1	The second dose is essential to obtain to achieve appropriate immunity against the disease	**8.6%**	**60.5%**	26.1%	3.4%	1.4%
	3.2	There is a specific time duration to give the second dose	**11.1%**	**59.6%**	25.6%	2.9%	0.5%
	3.3	There is the different specific time duration of booster doses of different COVID-19 vaccines	**6.1%**	**51.0%**	34.9%	7.5%	0.5%
	3.4	It is not possible to obtain the booster dose from another manufacturer or different platform vaccines	**5.7%**	**39.9%**	40.6%	11.6%	2.3%
Side effects of COVID vaccines	4.1	The commonly reported side effects of vaccines are fever, weakness, headache, and joint pain	**7.9%**	**55.3%**	27.0%	8.4%	1.4%
	4.2	The side effects can last for 4-5 days	**6.3%**	**52.8%**	29.9%	9.5%	1.4%
	4.3	The vaccine could have rare severe adverse reactions	**5.2%**	**52.4%**	33.8%	7.7%	0.9%

Overall, the participants had a high level of awareness of the importance of COVID-19 vaccination, eligibility of COVID-19 vaccines, dosage of COVID-19 vaccines, and side effects encountered after the vaccination (Table 2).

Conclusions and Recommendations

The majority of the study population, who represent the adult working community in the Galle district, had accepted the COVID-19 vaccination. Overall, awareness of the study participants on COVID-19 vaccines was satisfactory.

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CONVENTIONAL TREATMENT AND ONCOLYTIC VIROTHERAPY FOR TRIPLE NEGATIVE BREAST CANCER TREATMENT

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Introduction

In women under the age of 40, breast cancer is the leading cause of death whereas Triple Negative Breast Cancer (TNBC) becomes the most aggressive sub-type as well as the breast cancer type with the lowest survival percentages of all breast cancer types where it tends to represent about 15-20% of breast cancers worldwide where the treatment options are limited with low survival rates during past two decades [1]. TNBC is considered a highly aggressive subtype of breast cancer which is lacking the expression of progesterone receptors (PgRs), expression of estrogen receptors (ERs), and human epidermal growth factor receptor type 2 (HER2) where the prognosis of this cancer type is so poor due to the inheriting aggressive behavior as well as due to the lack of molecular targets for the desired therapy. In general, current ongoing treatments for breast cancers are cytotoxic, immunotherapeutic, and hormonal categories where the efficiency is low and where advanced stages develop resistance to these treatment methods [4]. Oncolytic Virotherapy (OV), which tend to be tumour specific found to be more efficient than the conventional treatments for TNBC. This review is carried out in order to analyze the effect of OV over conventional treatments and to find the efficiency of each virus on TNBC treatment.

Materials and Methods

Interested resource materials were compared and analysed both quantitatively and qualitatively while composing the present review article.

Literature Sources and Searches

ResearchGate, Academia, Google Scholar, PubMed, and Elsevier were searched for the following keywords within the title. (Oncolytic Virotherapy or Oncolytic Viruses) of Triple Negative Beast Cancer. Articles that were published over the past 20 years were only selected as resources for the present review article.

Inclusion and Exclusion Criteria

Qualitative and quantitative analysis of the existing knowledge about the early diagnosis methods of TNBC and the Oncolytic Virotherapy treatment in TNBC and the overall knowledge which can contribute to the development of future protocols were obtained. Articles published before 2002, duplicated, unrelated, unavailable full text papers were excluded under the exclusion criteria. They were selected in to prevent being biased while selecting the research.

Data Screening and Extraction

All the necessary qualitative and quantitative data were extracted according to the inclusion criteria and all the irrelevant data were excluded according to the exclusion criteria and the final data were reviewed and analysed.

Results and Discussion

Since TNBC cancers are highly aggressive and rapidly growing radiation therapy would not be very effective in early stages of TNBC unlike in other sub types. In TNBC patients BRCA1 gene has a considerate amount of mutation therefore, if radiotherapy could be used in early stages BRCA-deficient tumour loci could be completely eradicated from breast and surrounded tissue decreasing the loco regional reoccurrences in TNBC patients. Therefore, in this regard, OV is more effective since it causes oncolytic cell death sparing normal cells. Herpes simplex virus 1 can be identified as an enveloped double stranded virus which contains more than 80 genes. Due to that, this virus became more flexible in target genes where it can be engineered as an Ov (oHSV-1). The γ^{1}34.5 gene was deleted to constrain HSV-1 replication to cancer cells, resulting in a transcriptionally targeted vector that could not replicate in neurons and R-LM249 treatment in mice showed no symptoms of toxicity, hindered HER-2 positive tumour growth [5].Additional alteration to the HSV-1 envelope's entry mediator glycoprotein gD allowed for retargeting to unique overexpressed receptors in tumours, such as the HER-2. G47, a separate oHSV, comprised multiple gene mutations that further restricted replication of breast cancer cells. Extra mutations in the ICP6 and α47 genes limited replication to dividing cells while increasing immune stimulation [2]. Human adenovirus, Tumour specificity is accomplished by using tumour or tissue-specific promoters, including such MUC1, PSA, or PS2, to drive adenoviral genes required for replication, enabling the oAds to replicate preferentially in tumour cells while avoiding normal tissues [5]. Hypoxia induces the expression of hypoxia-inducible factor-1 (HIF-1), where it binds to the hypoxia response element (HRE) and activates the transcription of target genes in tumour cells. Combining these specific promoters into dual-promoter constructs will improve virus targeting and treatment safety. Results showed that P55-HTERT-HRE-TRAIL can replicate in TNBC cells effectively and cause cell death at low concentrations while having minimal effects on normal cells. Increased TRAIL concentrations in cell supernatant and lysate were confirmed by ELISA and western blotting assays. However, TRAIL expression was only slightly enhanced in normal breast cells, indicating tumour cell specificity of TRAIL, which further explains why TNBC cells ended up dead but normal breast cells did not. Vaccinia virus, This recombinant virus (VG9-IL-24) ends up killing infected breast carcinoma cell lines while having no perceivable cytotoxic effect on normal cells. Its anti-tumour effect was tested in *in vivo* using an MDA-MB-231 xenograft mouse model. The mice given VG9-IL-24 had decelerated tumour growth and longer survival times, as well as a higher survival rate. Since MDA-MB-231 is a TNBC cell line, the results of this study

provided evidence for the potential use of vaccinia virus throughout TNBC treatment [3]. In vitro, GLV-1h153 infected, replicated, and killed all TNBC cell lines. Mean tumor volume in vivo was 22 (IT), 29 (IV), versus 245 mm(3) (control; P 0.002) two weeks after treatment. All harvested lymph nodes and organs showed no signs of metastatic cells five weeks after treatment. A recombinant replicating VSV was discovered to selectively attack, replicate in, and kill erbb2-expressing breast cancer cells. Subsequent research looked into VSV's ability to inhibit TNBC. Recombinant VSV (VSVd51) was tested for cytotoxicity in mouse as well as human TNBC cells, and its influence on anti-cancerous immune response was confirmed. VSV had a significant therapeutic action by recruiting NK cells and CD8+ T cells.

Conclusion and Recommendations

TNBC is a highly aggressive form of breast cancer which lacks PgRs, ERs, and HER2. Since the conventional treatments found to be less effective in the later stages of the disease, OV can be implemented successfully since they are tumour specific. These OV have the unique property of using transcriptional as well as transduction targeting mechanisms, which limit the replication of oncolytic vector designs in cancer cells while sparing normal cells from harm. However, in order to determine whether certain OVs are also successful in human models, additional research is required in light of the above mentioned data and conclusions. In conclusion, the use of OV in the treatment of TNBC is efficient since it can lengthen the prognosis of this extremely aggressive heterogeneous illness in comparison to standard therapies like mastectomy and chemotherapy, which are less efficient in the latter stages of TNBC. As a developing nation, Sri Lanka now uses conventional therapies for TNBC, a type of cancer with exceptionally low survival rates upon diagnosis because of the aggressive nature of the disease. However, if properly applied, this OV approach will be a more effective treatment method for TNBC patients because it can completely eradicate the tumor while sparing normal cells without harming them, and it will lengthen the patients' life expectancy without causing any unfavorable side effects after the treatment. Therefore, it could be suggested to perform clinical trials to examine the accuracy of this Virotherapy against cancers.

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PARENTAL MENTAL HEALTH DURING COVID-19 PANDEMIC IN SRI LANKA: An Islandwide Survey

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Introduction

The rapid spread of COVID-19 has had a tremendous impact on human well-being. Owing to the lack of therapeutic treatments and immunizations, the unpredictability of the epidemic predicament, and the execution of a number of public health measures exacerbated the scenario, eventually putting immense effects on people's physical and mental health [1]. Physical isolation has led to a sedentary lifestyle, which is pathetic to many socialized communities, for instance, a Canadian based study conducted by Gadermann et al. [2] have reported that parents are more prone to, more emotional responses, also felling of suicide or self-harm. In their study, the authors further emphasized that such factors directly negatively affect children's lives. In another study, an Australian research group prioritized by Westrupp et al. [3] highlighted the worsening impact on children due to parent depression, anxiety, stress and irritability. A study by Thomson et al [4] also paramount those deteriorated social interconnections, where immediate interferences are needed.

Sri Lanka also among the nations hardly beaten by COVID-19, yet not recovered fully. It is important to understand the impacts of COVID-19 on parents in Sri Lanka, as there are only a limited number of studies carried out [5]. Under these circumstances, this study mainly aimed to understand the state of mind of Sri Lankan parents, who are having children at the level of kindergarten to collegiate. This work was carried out as a part of our project entitled 'Parental perception of homeschooling and distance learning during COVID–19 pandemic'.

Materials and Methods

The quantitative research approach was used in this study. The study was conducted during the third wave of COVID-19 in Sri Lanka from July to August 2021, when travelling restrictions in the country were implemented and schools were fully closed. Snowball sampling method was used to collect the data, 'Google Forms' (Semi-structured questionnaires in three languages—Sinhala, Tamil, and English) distributed through personal mail and social media, in order to reach the maximum number of participants island-wide within a short period. Following the collection of background data (e.g., demographic information), to assess mental health, PHQ-8 (Patient Health Questionnaire) depression scale was used. Initially, questions were given to understand the respondent's pre-mental

illnesses or who is under treatment and further responses from such participants were not considered for the analysis. In this PHQ-8 criteria, questions were included, i. thought of whether my life is meaningful, ii. trouble of concentrating on daily activities, iii. feeling bad about yourself, iv. poor appetite or overeating, v. feeling tired, vii. long sleeping, viii. feeling hopeless, and ix. little interest or pleasure in doing things. Parents were asked about their experience about the aforesaid criteria for two weeks. Additionally, questions were further given to the respondents to answer, in order to understand the experience of such mental conditions aggregated or initiated during the COVID-19 period or before. Moreover, enough space was given to respondents to include additional comments, such as their attempts to overcome such mental conditions. All over, responses from 587 participants were used for analyses after eliminating the irrelevant participants (e.g., having mental illnesses, already stressed before the COVID-19 pandemic), contradictions and incomplete answers. The statistical analysis was done using descriptive statistics and cross-tabulation. All the statistical analyses were performed by using the IBM SPSS statistics-23 software package.

Results and Discussion

The results show that the majority of respondents (about 40%) suffer from moderate-level depression, while just a little above a quarter (about 29%) show moderate-severe depression. A small proportion of respondents also suffer from severe depression (Figure 1).

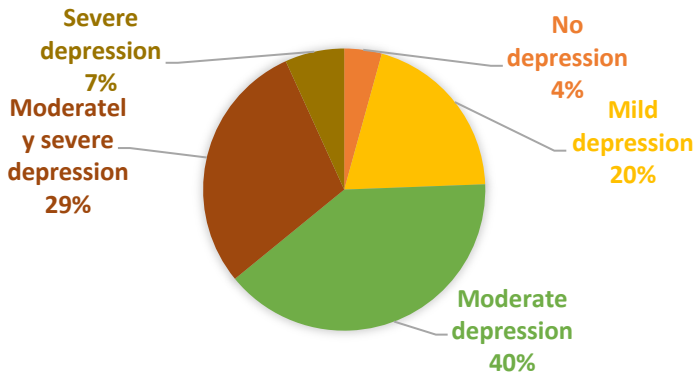

Figure 1. Depression level of parents during COVID-19.

The majority of respondents indicated that they are feeling tired even though they carried out small activities. The trouble of concerning daily activities such as cooking, cleaning and teaching their children and feeling hopeless are the other ranked responses (Figure 2).

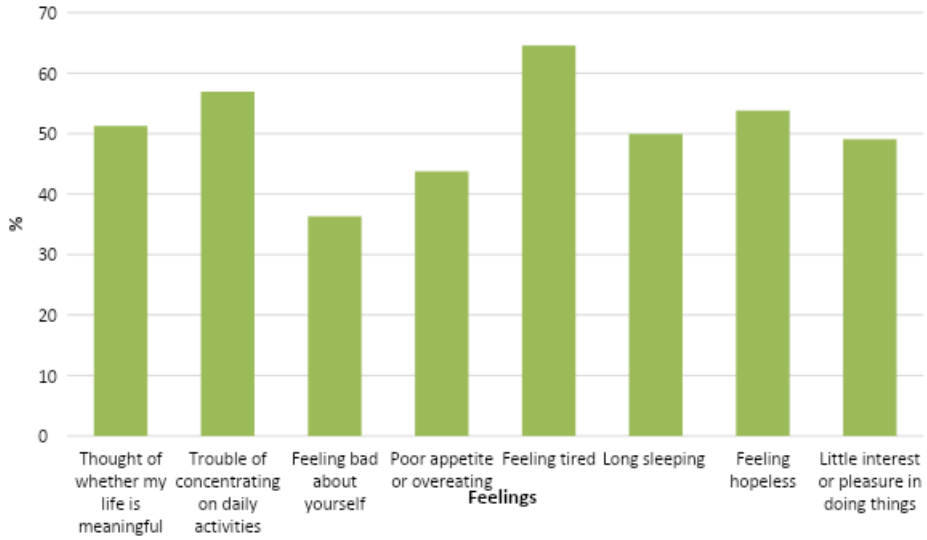

Figure 2. Percentage of various types of depression experienced by respondents

According to the Manova results, there are no significant differences among the respondents, based on age, gender, education, residential place, though the state of employment and the family income show a significant variation. Further, cross-tabulation results indicated that loss or reduction of income affected a lot for the parent's mental health. A considerable number of parents also worry about their children's education as it is disturbed a lot owing to the continuous lockdown and the movement restriction. On the other hand, self-isolation gives lots of pressure where the respondents missed to meet their favours. About seven percent of respondents were also infected with COVID-19 and some of the respondents stated that they are not physically fit like before and take those into their minds and concerning a lot about that, ultimately depressed emotionally.

Conclusions and Recommendations

In conclusion, the majority of respondents are suffering from a moderate level of depression, where the loss of a job or income due to the COVID-19 pandemic is the one of major concerns to them. In addition, they are also worried about children's education, as they could not continue properly. Further, our study also sheds light on the importance of well-being of parents as it directly affecting to adolescents, therefore quick alleviation is needed. Of this, according to the respondents forwarding towards the religious or spiritual aspects, collaborative family activities, virtual meetings with other relatives who are living within distance, and engaging in hobbies/exercise would be helpful to mitigate such depressions.

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SCREENING OF ENDOPHYTIC FUNGI FROM A MANGROVE PLANT; *Xylocarpus granatum* FOR ANTIMICROBIAL ACTIVITY

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Introduction

The increased prevalence of "ESKAPE" pathogens; *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and species of family *Enterobacteriaceae,* along with multidrug resistance has led to antimicrobial resistance crisis (AMR) in the human population [1]. The discovery of antimicrobial compounds from previously unknown microorganisms or known microorganisms producing novel bioactive metabolites from extreme habitats has become a new spotlight in drug discovery programs.

The mangrove ecosystem is well-characterized by a unique endophytic microbial diversity that is adapted to moderate saline and fluctuating environmental conditions. Endophytes are an endosymbiotic group of microorganisms that may have a part or all of their life cycles within their host while maintaining a complex but stable relationship without causing any apparent harm and are well-known for the production of secondary metabolites with medicinal importance.

In this preliminary study, endophytic fungi from leaves, twigs, and roots of a minor mangrove component native to Sri Lanka, *Xylocarpus granatum* J. Koenig (Family *Maliaceae)* were isolated, and different solvent extracts were screened for their *in vitro* antimicrobial activity against five selected human pathogens and an attempt was taken to detect and characterize promising antimicrobial compound/s by Thin Layer Chromatography (TLC) fractionation, coupled with contact-bioautography and Gas Chromatography-Mass Spectrometry (GC-MS).

Materials and Methods

Sample collection and isolation of endophytic fungi

Leaves, twigs, and roots of *Xylocarpus granatum* (National Aquatic Resources Research and Development Agency (NARA), Mangrove Reserve, Kadolkele, Negambo, Sri Lanka; 7.195676° N; 79.847247°E; 6 m) were collected and they were subjected to surface sterilization [2] and transplanted in Starch Casein Agar (SCA) and Potato Dextrose Agar (PDA).

Preliminary screening for in vitro antimicrobial activity by co-culture technique

Pure cultures of the isolated endophytic fungi were tested against five selected human pathogens; *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC

25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), and *Candida albicans* (ATCC 10231) for their *in vitro* antimicrobial activity by agar plug co-culture technique on Muller Hinton Agar (MHA). Suspensions of the test human pathogens were prepared in sterile saline water adjusted to have 0.5 McFarland Standard [3]. Antimicrobial activity was detected by the presence/ absence of a Zone of Inhibition (ZOI).

Extraction of metabolites from the selected endophytic fungus; PDA T.001
Based on the preliminary screening, the fungus; PDA T.001, was selected for the extraction of metabolites. Sequential extraction into hexane, ethyl acetate (EA), methanol, and distilled water (with increasing polarity) and direct extraction into ethyl acetate (dFEA) was also carried out using 21-days old, plate cultures of the fungus. Each extraction was triplicated, all three extracts were pooled, evaporated in an Angular Rotary Evaporator (Bibby RE200, United Kingdom) at 40-45^{0} C, the residue was weighed, and dissolved in a known volume of the respective organic solvent.

In vitro antimicrobial activity of solvent extracts of endophytic fungus; PDA T.001
In vitro antimicrobial activity of the crude organic extracts of the fungus was evaluated by the Standard Kirby-Bauer Disk Diffusion method [3]. The results were analyzed using Clinical Laboratory Standard Institute (CLSI) interpretative data for Antimicrobial Susceptibility Testing (AST).

Thin Layer Chromatography-Contact Bioautography
The crude fungal EA extract from sequential extraction (C-FEA) was fractionated by TLC (developed in hexane: EA – 1: 4) and visualized under UV and using TLC reagent; iodine vapour. Contact bioautography [4] was performed with selected human pathogens to detect the active fraction.

GC-MS analysis of the EA extract of plate culture extract (FEA) of PDA T.001
The C-FEA extract of PDA T.001 was analyzed by GC-MS with slight modification according to the method described by Tapfuma *et al.,* 2020 [5]. One mg ml^{-1} C-FEA in HPLC grade EA, the silica spot corresponding to the active spot of preparative TLC (pTLC-FEA) based on the contact bioautography, and the C-FEA spiked with pTLC-FEA (S-FEA) were analyzed by a GC-MS system; Agilent Technologies 7890A GC couples to a 5975C MS Triple-Axis Detector with an autosampler (Agilent 7693). The carrier gas was Helium with a flow rate of 1 ml min^{-1}. One microliter sample was injected in a split mode and the split ratio was 100: 1. The inlet temperature was 260^{0} C. The compounds were identified based on the comparison of their GC relative retention time and mass spectra with those of the National Institute of Standards and Technology (NIST) MS Search Library Software version 2.3.

Statistical Analysis

Minitab 16.1 (Minitab Inc, USA) was used and the results were expressed as the mean ± Standard Deviation value (SD). The values were compared using a two way t-test and one-way ANOVA. The probability value of p less than 0.05 ($p < 0.05$) was considered to be statistically significant.

Results and Discussion

Although no endophytic actinomycetes were isolated, 14 morphologically different endophytic fungi could be isolated from leaf, twig, and root into SCA supplemented with 50 µl ml^{-1} Nystatin and 50 µl ml^{-1} Nalidixic acid, which is a selective media for the isolation of endophytic actinomycetes. As the antifungals did not inhibit the growth of endophytic fungi at the recommended concentration and neither promoted the growth of actinomycetes, the concentration of the antifungal was increased from 50 µl ml^{-1} to 100 µl ml^{-1} gradually. However, it was still unable to inhibit the growth of endophytic fungi in the medium, indicating that the isolated endophytic fungi may be highly resistant to the antifungals used.

All fourteen endophytic fungi exhibited antibacterial activity against at least two test human pathogens while 10 isolates exhibit antifungal activity against *C. albicans* in the agar plug co-culture method. The isolate, PDA T.001 was selected for the secondary screening of metabolites as it showed both antibacterial and antifungal activity against all the five tested human pathogens and it was one of the two fungal isolates which showed antibacterial activity against *K. peumoniae* which is a multidrug-resistant Gram-negative bacterium.

In sequential extraction, only the ethyl acetate (C-FEA) extract exhibited antibacterial activity against *B. cereus*, *S. aureus*, and *E.* coli in the Kirby-Bauer disk diffusion method. This indicates the absence (or presence of lower amounts) of non-polar or highly polar compound/s with antimicrobial activity in the crude fungal extract. Direct extract of fungal metabolites into EA (dFEA) also inhibited the same test pathogens but to a lower extent and the ZOI was significantly lower ($p < 0.05$) compared to that of C-FEA fraction although the same concentrations were applied (20 µl from 1 mg ml^{-1} suspension) (Table 1). This may be due to the removal of any inhibitory compound/s into the hexane fraction during the sequential extraction which unmasks the antimicrobial compound/s present in the C-FEA fraction into their active forms. Although the agar plug co-culture test showed inhibition of *K. pneumoniae* and *C. albicans*, the FEA fraction of PDA T.001 was unable to inhibit the same two pathogens. This may be due to the C-FEA fraction containing a low quantity of antimicrobial compound/s responsible for the inhibition of these two pathogens or due to a synergistic effect of two or more active compounds which may be absent in other organic fractions.

Table 1. *In vitro* activity of EA fraction from sequential extraction (C-FEA) and direct extraction (dFEA) of endophytic fungus, PDA T.001, assessed by Kirby - Bauer disc diffusion

Organic extracts of PDA T.001	Mean diameter (mm) ± SD of ZOI		
	B. cereus	***E. coli***	***S. aureus***
C-FEA	13 ± 0.9[a]	19.3 ± 0.6[a]	19.2 ± 0.5[a]
dFEA	8.6 ± 0.5[b]	13.2 ± 0.4[b]	10.4 ± 0.2[b]
Positive control	23.0 ± 0[c]	19.5 ± 0.4[a]	30 .0 ± 0[c]

Data represented as mean ± SD (n = 3). The ZOIs were interpreted according to the CLSI Interpretative Standards of zone diameter for disc diffusion (CLSI M100 – Ed30, 2020). CLSI zone diameter breakpoints for 30 µg Chloramphenicol disk were ≥ 18 (susceptible), 17 – 13 (intermediate), and ≤ 12 (resistant), and 10 µg Gentamycin disk were ≥ 18 (susceptible), 17 – 13 (intermediate), and ≤ 12 (resistant). Mean values superscripted by different letters were significantly different at $p < 0.05$.

The TLC - contact bioautography showed a single inhibition zone, R_f of 0.49 against *B. cereus, S. aureus,* and *E. coli*, which were tallied with the spots (same R_f values) developed when the plates were treated with iodine vapour and visualized under UV_{254}.

The GC-MS profile of C-FEA spiked with pTLC-FEA; the S-FEA fraction gave one peak with a matching percentage of 99% and the corresponding compound was Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl. The same compound gave the highest matching percentage of 96% and 98% respectively for C-FEA and pTLC-FEA active spot. After spiking, the corresponding area for this compound/s increased by three folds, indicating that it was present in large quantities in the active spot of pTLC-FEA and that the corresponding retention times were also identical (Retention time – RT - 21.516 minute) (Figure 1).

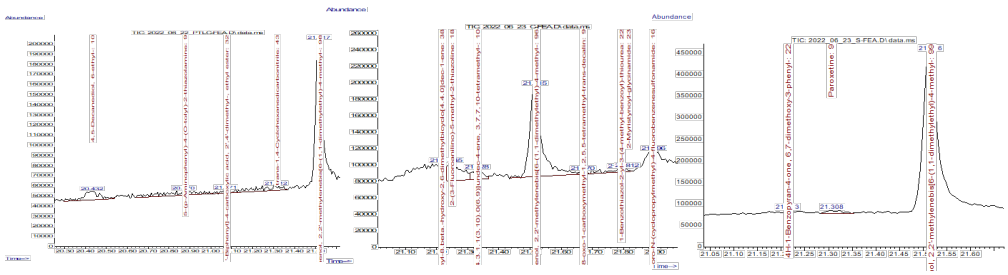

Figure 1. GC-MS profiles for different fungal ethyl acetate fractions of endophytic fungus, PDA T.001 from *Xylocarpus granatum.* **a.** GC-MS profile of pTLC-FEA fraction corresponding to Phenol 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl (RT – 21.517 minute) **b.** GC-MS profile of C-FEA fraction corresponding to Phenol 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl with a 96% matching percentage (RT – 21.515) **c.** GC-MS profile of the C-FEA spiked with the pTLC-FEA fraction (S-FEA) corresponding to Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl with a 99% matching percentage (RT – 21.516 minute).

Conclusions and Recommendations

A large number of endophytic fungi were associated with the native mangrove plant, *X. granatum,* 14 isolates out of 32 explant segments. All 14 isolates showed antimicrobial activity at least against two human pathogens tested and 10 isolates showed antifungal activity against *C. albicans* in preliminary screening using the agar plug co-culture method. The isolate, PDA T.001, could inhibit all five test human pathogens tested in the preliminary co-culture assay. However, in the sequential extraction of fungal metabolites, the FEA could only inhibit *B. cereus*, *S. aureus,* and *E. coli* in the Kirby – Bauer disc diffusion test. As determined by ZOIs, metabolites extracted directly into EA (dFEA) show a significantly ($p < 0.05$) decreased antibacterial activity compared to those extracted to EA using sequential extraction. The TLC - autobiography shows a single active spot (R_f = 0.49) against the pathogens tested indicating that the EA fraction contains bioactive compound/s with antimicrobial properties. The GC-MS analysis coupled with mass fragmentation analysis showed that the active spot observed in the TLC- bioautography was composed of a mixture of compounds responsible for the antimicrobial property of PDA T.001. The GC-MS profiles showed the highest matching percentage for Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl in all three C-FEA, pTLC-FEA active spot, and S-FEA which is a phenolic compound known to have antioxidant properties. In addition to established antioxidant activity, some phenolic compounds exhibit significant antibacterial activity. However, further investigation is needed to confirm the compound/s in PDA T.001 which is/ are responsible for the antibacterial activity for the tested human pathogens.

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DOSIMETRY OF ELECTRON BEAM THERAPY: VERIFICATION OF USING CC13 CYLINDRICAL IONIZATION CHAMBER

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Introduction

In medical Physics, Dosimetry is used extensively for radiation protection and is routinely applied to monitor the patient dose. Radiation Dosimetry continues to improve the accuracy of calibrating electron beam of radiation therapy. In this study, we investigate the feasibility of using a cylindrical chamber (CC13) instead of using parallel-plate chamber (PPC40) to be used in electron Dosimetry. Various international Dosimetry protocols recommend the use of a parallel plate ionization chamber in electron beam calibration. However, there should be another alternative to calibrate and measure the electron outputenergy in case of the parallel-plate chamber (PPC40) is unavailable.

Dosimetry parameters of electron beams:

In the analysis of clinical parameters of an electron beam, the depth dose curve for the megavoltage electron beams is employed. It gives the percentage depth dose with the depth in the given medium. This is useful in the treatment of superficial tumors and allows the sparing of the underlying tissue as a sharp dose drops off. The unique diametric characteristics of electron beam parameters are illustrated in figure 1 for the water medium.

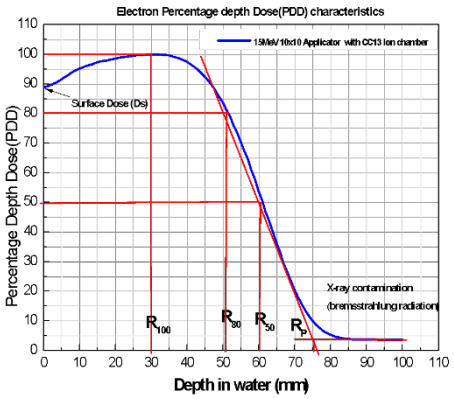
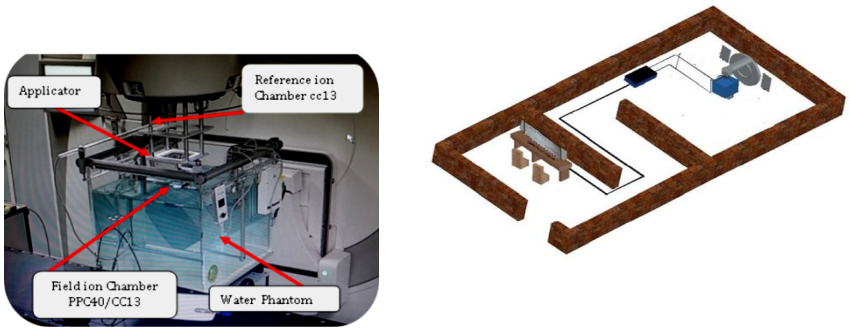


Figure 1. Show that the graphical representation of all electron beam parameters

Materials and Methods

The available nominal electron energies 6, 8, 10, 12, and 15 MeV were used in this study. The experimental setup is shown in Figure 2 (a). The experimental setup consisted of the Elekta Synergy platform linear accelerator, desktop computer, parallel plate (PPC40), cylindrical ion chamber (CC13), common control unit (CCU), water phantom (48 x 48 x 41 cm^3) and Omni-Pro-Accept 7.5 program.



(a) (b)

Figure 2. (a) Schematic diagram of the experimental setup of depth–dose measurements and the ion chamber setup and (b) conceptual diagram of the developed 3D radiation field analyzer for Dosimetry purpose.

All measurements were carried out according to the absorbed dose measurement Technical Reports Series No. 398.The Elekta synergy platform dual-energy medical linear accelerator was used to measure all measurements at the trial cancer treatment unit, in a base hospital in Sri Lanka. The electron energies of 6, 8, 10, 12, and 15 MeV were used with a 48 x 48 x 41 cm^3 water phantom. The distance between the collimator and the phantom face was 100 cm, Source to Surface Distance (SSD). The radiation dose was measured using both types of ion chambers namely parallel plate (PPC40) and cylindrical ion chamber (CC13).

The ion chamber was fixed at the water surface and aligned at the centre of the isocentre as shown in figure 2 (a). Temperature and pressure were measured. Ion chambers were subjected to a dose rate gradient of 600 MU min^{-1} and movement was also allowed in the z-direction in the water phantom. 6 × 6 $cm^2$and 20 × 20 cm^2 applicator sizes were used in this electron beam Dosimetry analysis. The percentage depth dose obtained for each 0.1 cm step of depth increase into the water from 0 – 11 cm. The ion chamber was remotely controlled from outside the linac control console room by the Omni-Pro-Accept 7.5 Software with a 25 m internet interface cable. The Percentage Depth Ionization (PDI) was obtained and converted to the PDD by Omini-pro software. Origin 8.5 software was used to acquire the percentage depth dose curves profiles. All measurements were subjected to a background subtraction that was obtained in the same radiation conditions but with the radiation beam turned off. The selected energies were treated from 0 cm to 11 cm depth range. Finally, the information R_{100}, R_{80}, R_{50}, R_P,

Surface dose, and X-ray contamination were obtained from the depth-dose curves for each electron nominal energies and particular applicator size for different types of ion chambers. The mean and most probable energies were calculated using the following equations.

$$E_o = 2.33R_o$$

$$E_{p,o} = 0.22 + 1.98R_p + 0.0025R_p^2$$

Where, R_{50} is the maximum dose fall off range, R_p is the Practical range.

Results and Discussion

The following percentage depth dose curves were obtained for different electron beam characteristics parameter values and both applicators, and ion chambers.

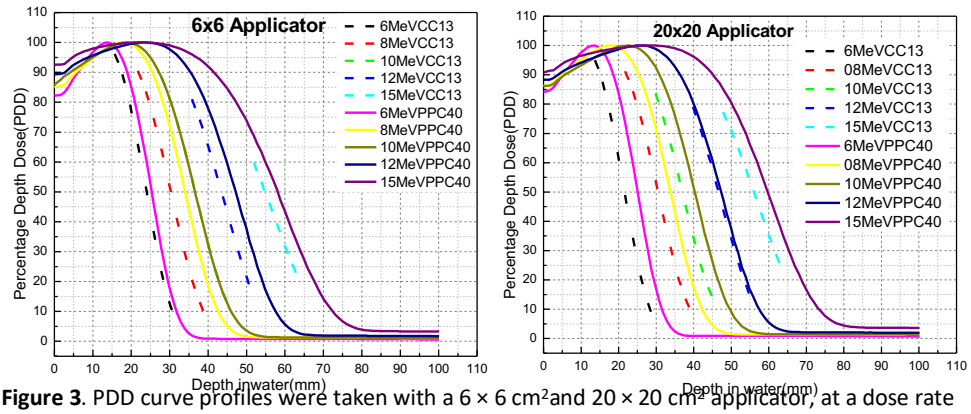


Figure 3. PDD curve profiles were taken with a 6 × 6 $cm^2$and 20 × 20 cm^2 applicator, at a dose rate of 600 $MUmin^{-1}$ for both ion chambers with different energies of electron beam

The PDD characteristic parameters have been analyzed for the clinical parameters, R_{100}, R_{80}, R_{50}, R_P, E_o, $E_{p,0}$ and tabulated in the table below.

Table: Comparison of percentage depth dose (PDD) curve characteristic parameters for (a) 6×6 cm^2 and (b) 20 × 20 cm^2 applicator by both types of ion chambers (PPC40 and CC13)

(a) For 6 X 6 Applicator

Energy	**6 MeV**		**8 MeV**		**10 MeV**		**12 MeV**		**15 MeV**	
Ion chamber type	CC13	PP40	CC13	PP40	CC13	PP40	CC13	PP40	CC13	PP40
D_{max}(cm) (R_{100})	1.27	1.37	1.47	1.77	1.87	1.87	2.17	2.3	2.17	2.22
R_{80}(cm)	1.96	2.08	2.46	2.81	3.02	3.02	3.60	3.99	4.45	4.72
R_{50}(cm)	2.38	2.51	3.03	3.39	3.65	3.66	4.34	4.71	5.46	5.80
R_P(cm)	3.81	3.20	3.85	4.24	4.67	4.61	5.36	5.83	6.86	7.24
X-ray contamination	0.53	0.78	1.09	1.28	1.72	1.77	2.16	2.67	3.73	3.67
Dose Gradient	2.07	2.22	2.17	2.34	2.23	2.30	2.40	2.43	2.28	2.29
E_o(MeV)	5.55	5.86	7.05	7.91	8.49	8.52	10.13	10.98	12.72	13.51
$E_{p,o}$(MeV)	6.36	6.58	7.88	8.67	9.56	9.39	10.91	11.85	13.93	14.69
Surface dose(%)	77.7	82.2	84.7	85	85.8	85.8	88.5	89.3	92.7	92.5

(b) For 20 X 20 Applicator

Energy	**6 MeV**		**8 MeV**		**10 MeV**		**12 MeV**		**15 MeV**	
Ion chamber type	CC13	PPC40	CC13	PPC40	CC13	PPC40	CC13	PPC40	CC13	PPC40
R_{100} (cm)	1.37	0.96	1.77	1.47	2.27	1.97	2.58	2.58	2.83	2.680
R_{80}(cm)	2.07	1.73	2.80	2.49	3.42	3.08	4.00	3.96	5.96	5.59
R_{50} (cm)	2.50	2.16	3.38	3.04	4.00	3.69	4.73	4.65	5.96	5.59
R_p (cm)	3.68	2.86	4.24	3.99	5.04	4.65	5.78	5.78	7.42	7.01
X-ray contamination	1.04	0.53	1.35	1.16	1.54	1.54	2.04	1.96	3.67	3.36
Dose Gradient (G)	2.21	1.95	2.32	2.02	2.44	2.37	2.60	2.45	2.39	2.38
E_0 (MeV)	5.83	5.04	7.88	7.09	9.32	8.59	11.03	10.84	13.9	13.03
$E_{p,0}$(MeV)	6.52	5.89	8.66	8.15	10.25	9.48	11.75	11.75	15.04	14.21
Surface dose (%)	84.1	84.5	86.1	85.4	86.3	85.7	88.3	85.1	90.8	91.2

Conclusion

The outcome of the study emphazises that it is possible to replace the parallel-plate chamber with the cylindrical chamber for high energy electron Dosimetry. The cylindrical ion chamber is showing ion collection efficiency better than that of the parallel plate ion chamber at a given dose rate of 600 $MUmin^{-1}$ for higher energy. The results further suggest parallel plate ion chamber is preferable for the energy range of the electron beam over the range of 6 MeV to 10 MeV Further this finding is useful in updating protocols for reference Dosimetry in linear accelerator electron beams.

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RELATIONSHIP BETWEEN BMI AND STATIC FOOT POSTURE AMONG THE PHYSIOTHERAPY UNDERGRADUATES OF FACULTY OF ALLIED HEALTH SCIENCES, GENERAL SIR JOHN KOTELAWALA DEFENSE UNIVERSITY

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Introduction

Foot biomechanics play a significant role in the quality of standing and ambulation. Foot problems are frequent and approximately found in 1 in 5 people in the community and are associated with mobility impairment and decreased quality of life. Body Mass Index (BMI) is a simple index which is interpreted using standard weight status categories and appears to be strongly correlated with various metabolic and other diseases. As the deviations of BMI from normal range has a significant effect on different types of foot posture and foot's loading characteristics that can lead for alterations of foot structure which may cause long term debilitating effects that impair quality of life. Minor alterations in the body composition may influence in static foot posture. The identification of factors such as BMI and alterations in static foot posture could lead to the development of better directed intervention strategies and potentially improve clinical outcomes. Less evaluations have been done in analyzing static foot posture measures with BMI in young adults. The aim of this study was to determine the association between BMI and static foot posture among the physiotherapy undergraduates of Faculty of Allied Health Sciences, General Sir John Kotelawala Defense University (FAHS, KDU). In our point of view, focusing attention on investigating the relationship between BMI and static foot posture among young adults seems essential.

Body Mass Index (BMI)

BMI is a simple index of weight for height which classifies underweight, overweight and obesity in adults (Table 1). It is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2) .

BMI= weight (kg)/height^2(m^2)

Table 1. WHO Asian BMI classification (Sharma et al., 2016).

Classification	BMI
Underweight	< 18.50
Normal range	18.50-22.99
Overweight	23.00-24.99
Obese I	25.00-29.99
Obese II	>30.00

Foot posture

Foot posture is generally defined by the contour of the medial longitudinal arch and can be categorized as normal (rectus), low-arched (pes planus) and high-arched (pes cavus).[1]

Foot posture index (FPI)

FPI is a clinical tool which has designed to provide a fast, simple, and multidimensional assessment of the foot type and is one of validated foot posture measurers that reflect the accurate static alignment of foot. As described by [2] the FPI consists of values obtained for six assessment criteria such as,FPI-I talar head palpation,FPI-II comparison of the curves above and below the lateral ankle malleolus, FPI-III calcaneal frontal plane position, FPI-IV prominence in the region of the talonavicular joint, FPI-V congruence of the medial longitudinal arch, FPI-VI abduction/adduction of the forefoot on the rear foot. Criteria were scored using whole numbers ranging from -2 to +2. The total score ranged from -12 (maximal supination) to +12 (maximal pronation) [2]. FPI scoring 0 to +5 depicts normal foot posture while +6 to +12 (pronated foot) and -1 to -12 (supinated foot) will depict potentially a pathological view.

Foot arch index (AI)

AI is a simple visual tool in assessing foot posture. Using AI foot posture can be simply categorised as high arch, normal arch, and low arch.(figure 1)[1].

Figure 1. Classification of foot posture (Menz et al, 2012).

Materials and Methodology

In this descriptive cross-sectional study, the data were collected from all the Physiotherapy undergraduates during the study period of 2021 of FAHS, KDU. 125

Physiotherapy undergraduates (96 females, 29 males) who completed the inclusion criteria were chosen as the study sample using nonprobability convenience sampling method. Ethical clearance was obtained from the Ethical Review committee of the Faculty of Medicine, KDU (RP/S/2021/40). Physiotherapy undergraduates who did not grant the written consent, were with fractures, dislocations, previous foot injuries and deformities, inflammation, neurological deficits, and muscle shortening in lower limbs, dysfunctions and deformities of spine, pelvis and lower extremities, with a recent reconstructive surgical history of lower extremities., with history of trauma and amputation in foot and with unstable physical and psychological status during data collection period were excluded from the study. Participants were informed through a sheet of information with all necessary details about the research which included aims, methodology, benefits, and limitations of the research in all three languages (Sinhala/ Tamil/ English). Validity and reliability of data collection tools were tested prior to the data collection via pilot study and students were recruited to the study followed by obtaining the written informed consent.
Static foot posture was evaluated by AI and FPI. Modified Harris Mat and AutoCAD version 22 were used in calculating AI. A standard stadiometer with digital weight scale was used to measure BMI. For the study analysis SPSS version 25 was used. Data collection procedure was conducted under following stations by 3 investigators. Prior to the data collection the 3 investigators were trained on the methodology under the supervision of a qualified physiotherapist and the validated instruments were tested for reliability. A single investigator collected the data in particular measurement to maintain the reliability of the data.

Station 1
A written informed consent was obtained from each participant through a consent form followed by an explanation about the study procedure. All the participants were provided with an information sheet, consent form and an interviewer administered basic assessment sheet according to their preferred language in Sinhala, English and Tamil. Each participant was given an identity number and all personal details were recorded under the identity number to protect the anonymity and confidentiality of the subjects throughout the research.

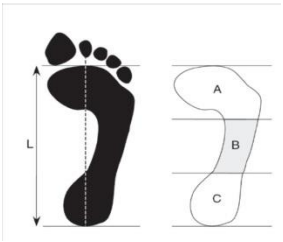
Station 2: Calculation of BMI
BMI was calculated using height and weight measurements using a standard stadiometer with the digital weight scale. Height and weight of each participant were measured in which height was measured in centimeters (cm) and weight was measured in kilograms (kg) using a standard stadiometer with a digital weight scale. Height measurements which were taken in centimeters (cm) were converted to meters (m). Three measurements of each parameter were taken and mean value of each was calculated separately. BMI for each subject was

calculated through the BMI equation using the mean values and was classified according to the BMI value obtained. (Table 1)

Station 3: Calculation of foot Arch Index

Participants were asked to stand in a relaxed position. Then they were asked to step in an ink pad (Harris mat) and then on to a white A4 paper. This was done for both feet. Then the foot prints were divided as the forefoot area (A), mid foot area (B), and the rear foot area (C) by separating into equal thirds by creating lines tangential to the axis line drawn excluding toes (Figure 2). Foot areas were calculated by the AutoCAD software version 22. Then the AI was calculated using the following equation and foot posture was categorized based on the reference values.Normal reference values of arch index are as follows [1].

Normal Foot arch index 0.21-0.26 ,Flat foot >0.26, High arch foot <0.21.



Arch index =B / A+B+C

Figure 2. Reference lines for calculating AI (Menz and Munteanu, 2005)

Station 4: Foot posture index measurements

All the students who participated were asked to stand barefoot. They were asked to take few steps and stand on a relaxed position with eyes looking forward. Both lower limbs were subjected to the assessment by the same examiner according to the criteria of FPI-6.

Data analysis and entry

The SPSS software version 25 was used for the data analysis in the study. Descriptive statistics were used in analyzing the demographic and physical characteristics of the study sample. The minimum, maximum, standard deviation (SD), mean, median, and skewness of the data were obtained. We considered the whole population as our study sample. Therefore, for further analysis parametric tests were used as the data followed a normal distribution. The Pearson correlation was used to assess the relationship of BMI and static foot posture.

Results and discussion

Results

The study sample consisted of 96 female and 29 male participants. The mean age of both female and male participants was 23 years (SD =2) with an age range of 19-27 in female population and 21-26 in male population. According to the foot posture classification of the left foot, 48.8% (n=61) normal foot, 10.4% (n=13)

supinated foot, 29.6% (n=37) pronated foot, 10.4% (n=13) highly pronated foot and 0.8 % (n=1) highly supinated foot. In the right foot, 53.6% (n=67) normal foot, 9.6% (n=12) supinated foot, 29.6% (n=37) pronated foot, 7.2% (n=9) highly pronated foot were obtained. According to the Foot Arch Index of left foot, 48.8% (n =61) normal foot, 11.2% (n = 14) high arc and 40% (n = 50) low arc foot. In right foot 53.6% (n =67) normal foot, 9.6% (n = 12) high arc and 36.8% (n = 46) low arch were obtained. In the study Pearson correlation test results were significant at $p<0.05$. According to Pearson correlation test results a significant positive correlation of 0.200 was observed between BMI and FPI in left foot with a significance of 0.025.A positive correlation of BMI and AI of left and right foot was observed with (p=0.012 r=0.225) and (p=0.041 r=0.183) respectively.

Discussion

In our study sample there were 76.8% (n= 96) female undergraduates and 23.2% (n= 29) male undergraduates. A similar study was done by Anjana Jayabandara *et al*.,2021 with 533 participants with female participants of 75.4% (n=402) and male participants of 24.6% (n=131). Our study sample was consisted of an age range between 19-27 with a mean of 23.34. The studies done by Anjana Jayabandara et al.,2021 on Prevalence of flat foot and its correlation with age, gender and BMI among undergraduates at FAHS, KDU and Alahmari *et al*.,2021 on Anthropometric determinants in healthy young adults have shown age ranges 19-26 and 18-25 respectively where the age ranges were similar with our study.

The BMI of our study sample was between 12.44 kgm^{-2} to 39.14 kgm^{-2} with a mean BMI of 22.2913 kgm^{-2}. Only 48% (n=60) individuals in the sample lie within the normal BMI range (18.5kgm^{-2}-22.99kgm^{-2}). Around 12% (n=15) were underweight and 20.8% of participants were in overweight category while 16.8% were in obese I category and 2.4% were in obese II category. These categorizations were done according to Asian BMI classification. This is comparable with the study findings of the other cross-sectional studies such as studies done by Shrama *et al*., 2016 and Sidhu and Tatla, 2002 where the prevalence of overweight and obesity were 12.7%,29.6% and 20% and 25.3% respectively.

For the measurement of foot posture, we have utilized AI and FPI. Studies which were done by [3], Aquino *et al*., 2018, Menz and Munteanu 2005., McLaughlin *et al*., 2016, Keenan *et al*., 2007 and [1] have given evidence on high validity and excellent reliability in FPI and AI.

Our objective was to find the relationship between BMI and static foot posture. Accordingly, a significant positive correlation between BMI, AI and FPI was obtained with $p <0.05$. There are lot of favorable studies which give positive correlations with BMI and Foot Posture such as studies done by [4],[5]and Carvalho *et al*., 2017.

Conclusion and Recommendations

Conclusion

In the relationship in BMI and static foot posture a positive correlation between BMI with FPI and AI was found. Accordingly, we observed that early detection,self-awareness about their current BMI and how their foot postures have been altered is essential in preventing future musculoskeletal disorders and improving quality of life.

Recommendations

Highly recommended to carry out a similar study with wide ranging sample size which represent the whole country as the sample size of the current study was limited.

Limited studies have been conducted with young adults. Therefore, more studies should be done in related with younger age group.

We recommend sharing our findings with wider population to raise awareness on the association between alterations in foot and BMI.

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EVALUATION OF ANTI-MICROBIAL EFFECT OF SKIN CREAM FORMULATED USING DIFFERENT COMBINATIONS OF SELECTED FIXED OILS.

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Introduction

Skin infections are a significant health issue in many underdeveloped and developing countries. These skin infections are treated with variety of synthetic medications. Microbial resistance to current medications is a serious public health problem worldwide that has sparked the interest in the research of alternative antimicrobial agents and formulations. Herbal fixed oils are enriched with variety of metabolites and fatty acids that have antimicrobial activity. Hence the purpose of this study was to formulate an antimicrobial skin cream using combination of fixed oils that can be used as an alternative to antimicrobial resistance problem.

Materials and Methods

Herbal fixed oils were selected based on a detailed literature search. Scientific databases such as PubMed, Google Scholar, Science Direct, Research gate and Scopus were used as the information source. All the articles published in English language which was related to antimicrobial effect of fixed oils were selected. Four fixed oils which have antimicrobial effects were selected based on the literature.

Antimicrobial activity of the four oil samples against three skin infecting pathogens, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 was determined by disc diffusion assay in Muller Hinton Agar (MHA).[1] The test was done in triplicate and results were recorded. The micro broth dilution method was used to obtain the Minimum inhibitory concentration (MIC) values of each fixed oil against all three pathogens.[2] Based on results of individual fixed oils, six combination ratios were decided. Individual fixed oils which showed more activity were incorporated into the combination in higher amounts. Antimicrobial activity of fixed oil combination ratios was done by agar disc diffusion assay, same as described in individual fixed oils. Different oil combinations were incorporated into discs instead of individual fixed oils. The test was done in triplicate and results were recorded.

Fixed oil combination ratios which showed highest activity against test pathogens were used to formulate creams. Oil in water (o/w) creams were formulated using heat method. [3] Skin cream formula was prepared based on the formula of aqueous cream without adding any preservatives.

Antimicrobial activity of formulated skin creams was evaluated by agar well diffusion assay in MHA. The cream formulations containing different concentration of the oils were diluted and samples were introduced into the each well. The diameter of zone of inhibition produced around each well was measured to indicate the degree of susceptibility of the test organisms to the sample. [4] Accelerated stability tests were performed at 40±0.1°C with 75% relative humidity (RH) up to one month period. Physical stability of formulations was inspected visually by color, homogeneity, consistency, and phase separation. [5]

Three creams were prepared by adding gentamycin into the skin creams formulated using three oil combination ratios (Ratio 1, Ratio 2, and Ratio 3) considering those creams as a cream base. Same was done with clotrimazole and three creams were prepared. The antimicrobial effect was then evaluated by agar well diffusion assay in MHA and compared with the market sample.

Statistical analysis was performed with SPSS. Data was presented as mean ± standard derivation (SD). ANOVA was used to check significant differences in mean of the parameters evaluated. Differences were considered to be statistically significant at a p value of < 0.05. [4]

Results and Discussion

Based on the literature review neem oil, black seed oil, virgin coconut oil and sesame oil were selected. All four fixed oils showed antimicrobial activity against all three test pathogens except, neem oil. Neem oil did not show an inhibitory effect against *P. aeruginosa*. Black seed oil showed an inhibitory effect of 50.33mm ±0.57 against *S. aureus* which was greater than the activity of positive control, 10 mg/ml gentamycin. Combination of fixed oils increased the activity against test pathogens. The formulated creams showed significant antimicrobial activities against all three pathogens but when compared to fixed oil combination ratios, activities of creams were lower than fixed oil combination ratios. Interestingly the activity against *C. albicans* was increased after incorporating clotrimazole into fixed oil cream bases. MIC values for neem oil, black seed oil, coconut oil and sesame oil against *S. aureus* were, 125 mg/ml, 62.5 mg/ml, 500 mg/ml and 250 mg/ml respectively and values for black seed oil, coconut oil and sesame oil against *P. aeruginosa* were, 500 mg/ml, 1000 mg/ml and 62.5 mg/ml respectively. MIC values for neem oil, black seed oil, coconut oil and sesame oil against *Candida albicans* are, 62.5 mg/ml, 1000 mg/ml, 125 mg/ml and 500 mg/ml respectively. The physical and stability study results were satisfactory.

Conclusions and Recommendations

Effective antimicrobial creams can be formulated combining neem oil, black seed oil, coconut oil and sesame oil that can be used as an alternative treatment for skin infections which will help to overcome antimicrobial resistance problem. This study can be further improved by studying release characteristics of creams, synergism activity of combining fixed oils and applying these creams into clinical isolates of microorganisms.

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DETERMINING QUALITY INDICATORS IN THE PRE-ANALYTICAL PHASE: A STUDY AT COLOMBO SOUTH TEACHING HOSPITAL, KALUBOWILA.

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Introduction

Laboratory investigations are basically carried out for patient management, therapeutic monitoring, preventive care and disease diagnosis [1]. With the advancement of science, patient healthcare becomes heavily dependent upon laboratory investigation reports. Colombo South Teaching Hospital (CSTH) is the second largest hospital in Clomobo District wich was upgraded to Teaching status in 1995.The hospital maintains a bed capacity of more than 1110 and it is only second to National Hospital Sri Lanka which is the largest government hospital in Colombo District. As a teaching hospital, it offers medical and surgical treatments for an array of pathological conditions and provides services including specialized areas such as Rheumatology, Endocrinology, Psychiatric and Neurology.There are two laboratories which used to cater different patient cohorts including Outpatient Department (OPD) Laboratory and routine Laboratory (Chemical pathology, Microbiology, Hematology, and Histopathology) which serves for inpatients.

Total Testing Process (TTP) and errors in pre-analytical phase

Entire process of laboratory performance starting fromtest ordering to report issuing is known as TTP [2]. TTP can be categorized into three phases which are known as pre-analytical, analytical and post analytical phase. According to the literature, pre-analytical phase is more error prone than others in TTP accounting for 70% of errors in TTP [3]. Quality performance of laboratory testing creates confidence in all stakeholders including clinicians, patients and laboratory staff. Thus, the international organization for standardization recommended that it is important that medical laboratories to be examined periodically to evaluate the TTP of laboratory.

Quality Indicators (QIs) and its use

Quality Indicators (QI) is a fundamental tool for quantify pre-analytical errors and helps to decide performance of current systems. Quality indicator is defiend as anobjectiveevalution of a patien's health care status such as patient safety,

efficiency, affectivity, timelines and patient centeredness. Therefore, QIs can be used to demonstrate the quality of the laboratory while helpingdecision making process and can be used to compare between and within the laboratory performance. The international federation of clinical chemistry and laboratory medicine (IFCC) had developed 16 specific QIs focusing on the pre-analytical phase [4].

Table 1. List of 16 QIs related to pre-analytical phase.

Quality indicator	Description
QI-1 Appropriateness of test request	Number of requests with clinical question (%)
QI-2 Appropriateness of test request	Number of appropriate tests with respect to the clinical question (%)
QI-3 Examination requisition	Number of requests without physician's identification (%)
QI-4 Examination requisition	Number of unintelligible requests (%)
QI-5 Identification	Number of requests with erroneous patient identification (%)
QI-6 Identification	Number of requests with erroneous identification of physician (%)
QI-7 Test request	Number of requests with errors concerning test input (%)
QI-8 Samples	Number of samples lost/not received (%)
QI-9 Samples	Number of samples collected in inappropriate containers (%)
QI-10 Sample	Number of samples hemolysis (hematology, chemistry) (%)
QI-11 Samples	Number of samples clotted (hematology. chemistry) (%)
QI-12 Samples	Number of samples with insufficient volumes (%)
QI-13 Samples	Number of samples with inadequate sample-anticoagulant ratio (%)
QI-14 Samples	Number of samples damage in transport (%)
QI-15 Sample	Number of improperly labeled samples (%)
QI-16 Samples	Number of improperly stored samples (%)

Although it is an important aspect to monitor the error rates in pre-analytical phase of the laboratory testing, thereare minimal literature onestablishing QIs suitablefor government hospital laboratories in Sri Lanka to monitor its performance. Therefore, this study aimed to determine suitable QIs for CSTH. This study was guided by specific objectives; to identify frequency of occurring pre-analytical errors identified in the specimens received to CSTH laboratories and to quantify frequency of predefined (IFCC) quality indicators and to select suitable QIs to monitor quality performance.

Materials and Methods

The errors identified during specimen acceptance at the OPD laboratory and Hematology and Biochemistry sections of the main laboratory were recorded in a log book during February to August 2022 after obtaining ethical clearance from Ethics Review Committee of CSTH. Considering feasibility of monitoring QIs, we selected 08 QIs from 16 QIs based on observations and experience of the investigators of this study while the other most frequent errors were also recorded. Accordingly, IFCC defined QIs number of samples illegible hand writing % (QIs 4), number of samples lost/not received % (QIs 8), number of samples collected in inappropriate containers % (QIs 9), number of samples hemolysis % (QIs 10), number of samples clotted % (QIs 11), number of samples with insufficient volumes % (QIs 12), number of samples damage in transport % (QIs 14), number of improperly labeled samples % (QIs 15) were calculated based on the records maintained at three laboratories mentioned above.

Analysis of the results was based on six Sigma metrics method in which error rate and defects per million was calculated using the formula below.

$$\text{Error rate} = \frac{\text{Observed total number of errors}}{\text{Total number of specimens}} \times 100$$

$$\text{Defects per million (DPM)} = \frac{\text{Observed total number of errors}}{\text{Total number of specimens}} \times 100{,}0000$$

Then the DPM value has been converted to a sigma value by using an online statistical table(http://www.westgard.com/six-sigma-table.htm) and according to the resulted in performance level laboratory performancewere categorized as below [5]. Sigma value ≥ 5 very good, 4 < 5 sigma good, 3< 4 sigma minimum, < 3 sigma unacceptable. According to the Six Sigma principle, QI performance was good and acceptable if Six Sigma value was >4. QI performance should be improved if Six Sigma value was 3-4, corrective action should be taken immediately if Six Sigma value was <3. Therefore, in this study suitable QIs were selected if sigma >4.

Results and Discussion

Specimen characteristics

During the six months of period from 22.02.2022 to 22.08.2022, we received a total of 809,146 samples from biochemistry laboratory, 90246 samples from hematology laboratory and 36,670 samples from OPD laboratory. For the Biochemistry lab, samples were received for mainly five types of tests such as General biochemistry, hormones, sugar, lipid profile and Troponin I. Specimens for Full Blood Count (FBC), coagulation studies, special tests were received for Hematology laboratory. For the OPD laboratory specimens were mainly received for FBC, Urine Full Report, Urine HCG, ESR and stool full report.

Identification of frequency of commonly occurring pre-analytical errors in CSTH according to quality indicators defined by IFCC.

Acccroding to the sigma values, the performance levels of each QIs are depicted below in Figure 1.1.a QI 4, 8, 9, 10, 11, 12, 14 and 15. When considering specimens received for OPD laboratory, there were a total of 13,618 pre-analytical errors (37.1%) identified out of 36,670 test requests. The highest error parentage (35%) was reported for QI 4 (number of eligible request form) while the second most error rate (0.87%) was reported for QI 11 which denotes number of samples clotted. QI 4 reported an unacceptable performance. While QIs 8, 9, 12 and 15 indicated good performance level, QI 14 found to record a very good performance level.

Among the specimens received for Haematology laboratory, the highest rate of error (16%) was reported with number of eligible handwriting (QI4) giving unacceptable performance followed by QI 12 Number of samples with insufficient volumes accounting for 6.2% of errors resulted in minimum acceptable performance. QI 8, 9, 10, 11 and 15 shows sigma value ranged in 4-5 suggesting good performance. QI 14 Number of samples damaged in transport found only with a 0.002% error indicating a very good performance.

In biochemistry laboratory too, the highest error rate was reported for QI 4 number of illegible hand writing resulting in unacceptable performance (9.1% errors). It was noted that the QIs 9, 10, 11, 12 and 14 indicated a high performance with low error parentages.

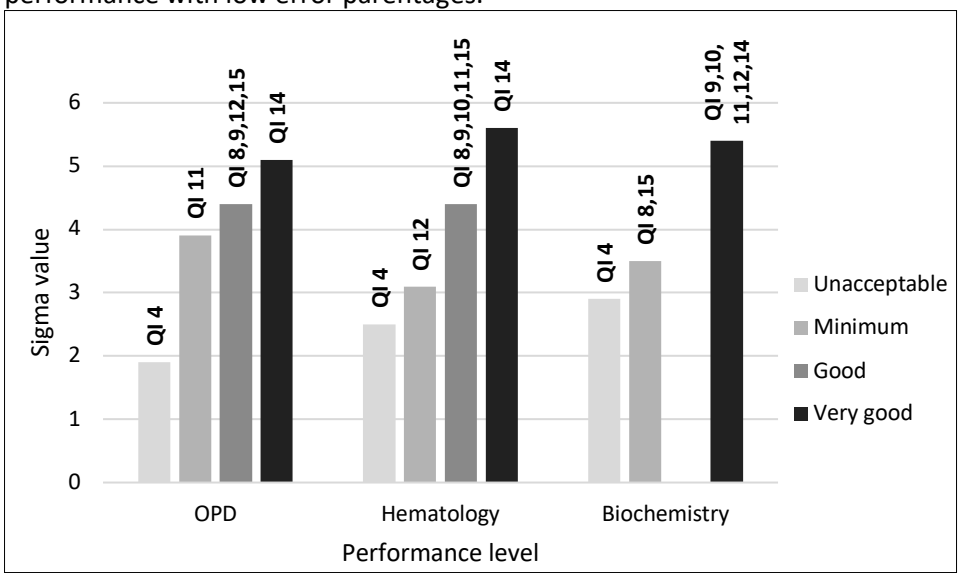

Figure 1.1a. Sigma value-based performance levels for specimens received to different laboratories based on selected QIs.

Identification of frequency of commonly occurring pre-analytical errors in CSTH other than quality indicators defined by IFCC.

The following were identified at CSTH laboratories as the most commonly reported errors and its performance levels. Ordering tests that are not performed on Sundays and public holidays (QIs A), not labeling urgent tests (QIs B), ordering same test for same person repeatedly (QIs C), incomplete request forms (QIs D), overfilled specimen collectiontubes (QIs E), not labeling as high risk sample in red (QIs F), inadequate size of the request form (QIs G).Overall, inadequate size of the request form shown to be a common problem in all three laboratories due to the highest error rates (11%, 29.7% and 44.5 % in OPD, Biochemistry and Hematology laboratories, respectively). Figure 1.1.b shows further information on the performance of each QI at three different laboratories.

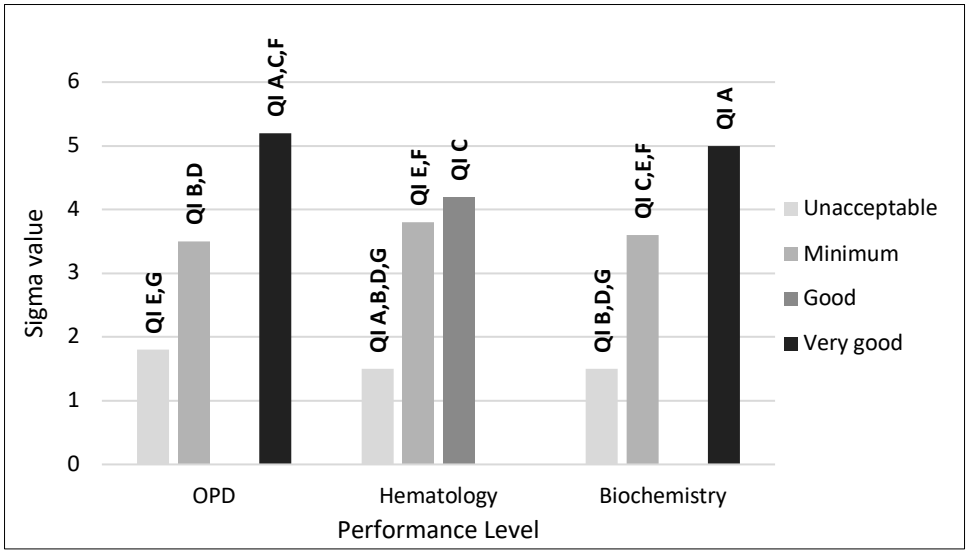

Figure 1.1b. Sigma value based performance levels for specimens received to different laboratories based on additionals QIs.

Overall, as per the results above, Illegible handwriting (QI 4) gives unacceptable performance in all three laboratories due to heavy workload and tight shipments. Moreover, inadequate size of the request forms, incomplete request forms, not labeling urgent tests identified to cause higher error rates in all aboratories. Usually, these errors can be minimized by use of either printed request forms or electronic requests utilizing bar codes for specimen labelling as suggested in previous research. All these would contribute in improving quality of care provided by the hospital.

Arranging annual training sessions, workshops and continuous professional development programs to introduce international guidelines for health care practices would also be recommended. Analysis of QI in pre-analytical phase

helps to create build up new strategies to implement quality system. Further studies are recommended to determine the importance and use of the other pre-analytical QI and to identify frequently reported other QI in CSTH laboratories. We also recommend to implement corrective and preventive actions for the errors with higher frequencies identified here.

Selection of QIs for CSTH

For all three laboratories, specimen damage during transportation could be used as a QI as it scored a very good performance. In addition, QI 8, 9, 12 and 15 can be used to monitor performance of pre-analytical phase activites of OPD laboratory. QIs 8, 9, 10, 11, 12 and 15 can be used to monitor performance of Hematology while only QIs such as 9, 10, 11 and 12 can be used to monitor pre-analytical activities of Biochemistry laboratory.

Further studies are recommended on frequently seen additional QI in CSTH as it is worth identifying effects of these errors. Following the guidelines of International Standalization Organaization (ISO) quality specification, pre-analytical phase needs continuous evaluation with performance of QI.

Conclusions and Recommendations

This study finding enabled us to select suitable QIs to monitor performance of pre-analytical phase of laboratories at CSTH. The study highlights that each laboratory has both specific set of QIs to be monitored in addition to the QIs identified to be suitable to monitor all laboratories. That is, number of samples collected in inappropriate containers and number of samples with insufficient volumes were found to report lower frequencies and can be used to monitor performance of all three laboratories of CSTH while there were other QIs which are able to monitor its performance. On the other hand, this study findings identified the areas for continual improvement following corrective and preventive actions to minimize errors which resulted in poor performance. In addition to the QIs defined by IFCC, we identified not labeling urgent tests (QIs B), incomplete request form (QIs D) and inadequate size of the request form (QIs G) as most commonly reported errors in CSTH laboratories. Analysis of QI in pre-analytical phase helps to create build up new strategies to implement quality system. Further studies are recommended to determine the importance and use of the other pre-analytical QI and to identify frequently reported other QI in CSTH laboratories. We also recommend implementing corrective and preventive actions for the errors with higher frequencies identified here.

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EXPLORATIONS OF PATIENTS' EXPERIENCES AFTER CORONARY ARTERY BYPASS GRAFTING

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Introduction

Coronary artery disease (CAD) is a leading cause of death worldwide including both in high-income and low- to middle-income countries [1]. It has been estimated that around 126 million individuals (1,655 per 100,000) are affected by CAD universally and it is approximately 1.72% of the world's population. Also, nine million deaths were caused by CAD globally [2]. Men were more commonly affected than women. According to recent estimates, the mortality rate from cardiovascular diseases in Sri Lanka was 524 deaths per 100,000 and this is higher than that observed in many high-income countries. Further, CAD is a leading cause of death in Sri Lanka [3].

Coronary artery bypass surgery (CABG) is the established form of treatment for coronary artery disease, and most CABGs are performed for multiple vessel disease. The main purpose of undergoing CABG is, to regain multi-factorial recovery of all the aspects of the patients. CABG is a type of surgery that improves blood flow to the heart. During CABG a healthy artery or vein from the body is connected or grafted to the blocked coronary artery. The grafted artery or vein bypasses (goes around) the blocked portion of the coronary artery. This creates a new path for oxygen-rich blood to flow to the heart muscles. CABG surgery is considered effective in relieving symptoms and reducing mortality. It is reported that the mortality rate of CAGB is relatively low, and it remains around 2 %–3 %, thus this surgery is widely practiced [4].

Although numerous studies have investigated the outcome of CABG operation, little attention has been given to post-CABG patients' experiences. A minimal number of literatures was found in the Sri Lankan context. Hence, it will be paramount important to explore the patients' experiences after undergoing CABGs in terms of improving the surgical outcomes of the patients. Therefore, this study aimed to explore patients' physical, psychological, and social experiences after CABG surgery.

Materials and Methods

Study Approach and Design

A qualitative descriptive approach and a phenomenological design were utilized in this study to explore the patients' physical, psychological, and social experiences after CABG surgery [5, 6].

Study Setting and Participants

The study setting was the cardiac ward of one of the largest tertiary care private hospitals. The participants of this study were patients who had Coronary Artery blocks and so, had undergone elective CABG for the first time at the cardiac unit. Eleven patients who were purposively selected were recruited for the study as it was the saturation point of the sample. Patients who had complicated health conditions such as diabetes mellitus, hyperlipidemia, hypertension, hypotension, infections... etc. and poor psychological well-being were excluded from this study.

Ethical Consideration

Ethical approval was obtained from the Ethics Review Committee of the private hospital and permission for this study was taken from the Director of Medical Services, and the Chairman of the hospital. Before starting the study, all the participants were fully informed of the study objectives. Prior to collecting the data, voluntary participation was encouraged, and informed consent was obtained from each and every participant. Anonymity, privacy, and confidentiality were ensured at all stages of the study by labeling the subjects with a specific code for collected data and securing the information only among the research team.

Data Collection

For data collection, semi-structured interviews were used since it provides a clear set of instructions for interviewers and can provide reliable, comparable Qualitative data. A theme list was used to guide the interviews, which was validated by referring to appropriate literature and expert opinions [5, 6]. Also, it was pre-tested to ensure reliability. The average time duration of interviews was 30 minutes. All the interviews were audio recorded while written notes were made on each patient manually. During the interviews, patients were separated from others and a calm and quiet environment was provided. Demonstrating good listening and encouraging them by demonstrating a positive affirmation of their viewpoints, and tested interview skills were used to conduct more effective interviews. Furthermore, close observation of the patients' facial expressions, body language, and other non-verbal clues such as sighing, and crying, was done.

Data Analysis

The data were analyzed using Colaizzi's method [7]. Interview recordings were carefully listened to get a clear idea of the whole content of participants'

explanations and views. The records were transcribed into texts with several reviews. A general impression of the structure of the experiences was obtained. Important statements which were relevant to the phenomenon were extracted from them and recorded on separate sheets. The meaning of each phrase was described and defined. Each description was then reread with a focus on identifying descriptive expressions in the exact language of the participants. Each descriptive expression was named and grouped with other common compatible descriptive expressions. The formulated meanings were categorized into sub-themes and then themes, and these categories were referred to initial protocols for confirming their validity.

Results and Discussion

In terms of describing characteristics of the study sample, the sample consisted of eight males and three females, and their age range was from 48 - 65 years. According to the ethnic distribution, the majority were Muslims (45.4%) and others were Sinhalese (36.4%), and Tamils (18.2%). Among them, 90.9% were married and 81.8% had children while the rest were living with their spouse or relations. Further, the majority had secondary-level school education (63.6%) and 27.4% were illiterate and 9% completed higher studies. The majority were retired or never had a formal occupation (90.9%) whilst 63.6% had a monthly income of less than 50,000 LKR and 27.4% had no income whereas 9% were with more than 100,000 LKR.

Four themes were derived from the study findings: Unbearable pain and discomfort of the body; Alterations in daily routines; Loneliness; and Problematic issues in engaging in social activities (Figure 1).

Unbearable pain and discomfort in the body

The patients after CABG were always suffering from pain mainly related to chest incision pain and leg/hand incision pain resulting from the surgery. Also, complaints of body aches and pain related to other invasive procedures. Further, they often suffered from fatigue due to undergoing the surgery and experience some breathing difficulties too. According to Schou & Egerod (2008), postoperative pain was the most prevalent symptom experienced by many patients. Also, swelling limbs, pain in the chest incision and leg incision, coughing, appetite disturbance, and bowel pattern disturbances are the most concerning experiences in the early recovery period after CABG.

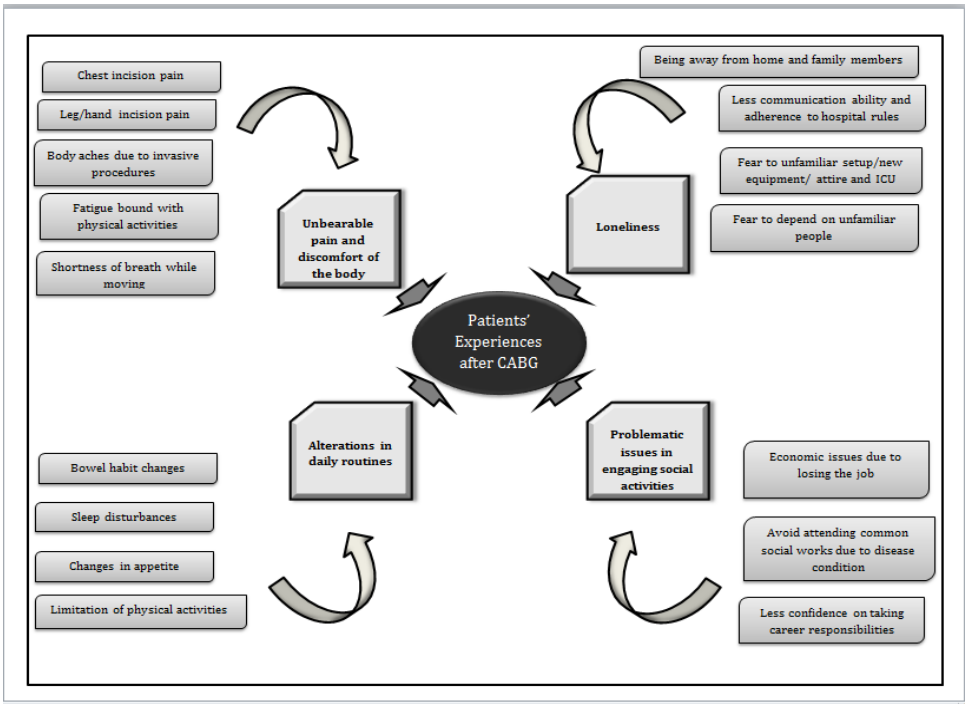

Figure 1. Patients' Experiences after CABG

Alterations in daily routines

According to the study findings, the patients had experienced that their physical routines had been changed because of changing their bowel patterns, losing their appetites, and due to changing their sleeping patterns.

Loneliness

The study results emphasized that the majority were suffering from loneliness due to several facts such as separating from the family and struggling with communication difficulties. In comparison to these findings, Schou & Egerod (2008) mentioned that some patients described the Intensive Care Unit (ICU) as unfamiliar and different from their usual way of being in the world. Despite communication difficulties, patients sought to reclaim their known world by connecting or re-engaging with their families in the ICU. However, a study done by Dunkley et al., 2007 declared visiting the ICU helped some patients to prepare emotionally for the procedure and this seemed to result in better post-operative recovery. The current study patients also agreed with this belief. Furthermore, they felt fear and uneasiness due to the unfamiliar environment, strange attires, equipment, and many invasive procedures in ICU. Also, they were anxious due to depending on unfamiliar people instead of family members.

Problematic issues in engaging in social activities

In the current study, under the patients' social experience after CABG, many of them worried because they will be unable to attend social meetings and actively participate in common social works because of the disease condition. Also, they worried because they were treated as severely ill patients by society. Further, most of them were breadwinners of the family and they were distressed about being unable to attend their jobs and will have to take compulsory leave due to the surgery. Some were less confident about continuing their carrier responsibilities. Furthermore, few participants revealed that they held higher positions in various social committees, however because of the disease, they could not actively participate in common activities. Moreover, they were unhappy regarding the image disturbance that resulted from the surgery as it will affect their family relationships.

Conclusions and Recommendations

CABG surgery had brought a series of challenging experiences to the patients' lives. Four themes related to experiences after CABG were derived from this study, and they are unbearable pain and discomfort of the body, alterations in daily routines, loneliness, and problematic issues in engaging in social activities. Located under these themes there are patients' experiences such as chest incision pain, leg/hand incision pain, body aches, fatigue, and breathing difficulties. Further, they experienced changes in their bowel patterns, appetite, and sleeping patterns. Furthermore, the patients experienced separation from their families, struggled with communication difficulties and were suffering from fear and uneasiness. Also, they were anxious due to depending on unfamiliar people instead of family members. Moreover, they are distressed because they will be unable to attend social meetings, actively participate in common social work, inability to attend their jobs, and take compulsory leave due to the surgery.

However, it seems that the patients are worried and become anxious due to inadequate awareness about the procedures. Some studies revealed that the outcomes of the surgery and recovery can be improved by enhancing patients' knowledge using educational tools. Therefore, it is recommended to organize awareness programmes at the pre-admission clinic to educate patients before CABG surgery and to allay their misconceptions. Further, it is recommended to develop a comprehensive teaching package to be used by all healthcare professionals to eliminate the inconsistent messages that will be received by the patients.

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FOCUS AREA

Information Communication Technology & Knowledge Services

AN APPLICATION OF BAYESIAN NETWORKS IN STOCK MARKET ANOMALY DETECTION

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Introduction

Stock exchanges are where secondary shares are issued to meet the capital requirements of listed companies, thereby ensuring, and enabling, the continuity of economic expansion. Beyond this basic purpose, stock markets enable investors to get higher returns on their investments. In practice, however, the extent to which individual investors can achieve such profits is a topic of constant debate among researchers and economists. The majority of investors view extraordinary stock market losses as mere coincidence often referred to as "just a bad day". Statistically, few investors seek to observe the causal relationship between trade timing and corresponding returns. What if there was a way to learn these connections from observations of the environment? Therefore, this study focuses on building a causal model to interpret stock market anomalies on the Colombo Stock Exchange (CSE).

Materials and Methods

Problem Analysis

Although the CSE plays an important role among global stock exchanges, there is little literature on behavioral analysis of the CSE. However, in order to understand the nature and behavioral patterns of the CSE, it is important to identify various economic theories and hypotheses applicable to the stock market. The Efficient Market Hypothesis (EMH) is such a theory that postulates share prices reflect all available information at all times and hence stocks or securities are always properly priced; entailing the market is efficient. A market anomaly is an unexpected occurrence that causes the performance of a stock or group of stocks to deviate from the assumptions of the EMH. These anomalies can belong to one of three types: Fundamental Anomalies, Technical Anomalies and Calendar Anomalies. This study mainly focuses on Calendar Anomalies and its detection. Calendar anomalies are market anomalies, which depicts a deviation in normal behavior of stock associated with various time periods of the calendar.

Across the years majority of the literature have used mainly two statistical techniques to determine the existence of anomalies within the market; Ordinary Least Square (OLS) Regression model [1][2][11][13]; while some literatures have additionally explored the Generalized Autoregressive Conditional Heteroskedasticity (GARCH) model to identify which method is better in that

particular context to detect the given form of anomaly [3][7]. Parametric methods such as Mann-Whiney U test as well as non-parametric methods such as Paired sample t-test [1] have also been applied to substantiate the existence of various market anomalies. The empirical results of these studies demonstrate the presence of seasonality in stock returns. A few such seasonal effects are namely, Day-of-the-Week effect, Month-of-the-Year effect, Turn-of-the-Year effect, Turn-of-the-Month effect, Holiday effect, etc. The application of Bayesian Networks for market anomaly detection can be seen in North and South American stock markets through which Teodoro and de Castro [12] investigated the anomalies of stock market trading within the Brazilian capital market (BOVESPA), New York Stock Exchange (NYSE) and the American NASDAQ stock exchange.

Despite the numerous literatures with regards to stock market anomalies, considerably limited amount of studies has been carried out through the employment of Bayesian Networks and additionally in the context of Asian markets including Sri Lanka, no such literature can be found. Therefore, this study attempts to fill the existing gap by modelling stock market anomalies of Colombo Stock Exchange through the use of Bayesian Networks.

Bayesian Networks uses probability theory as a foundation enabling the derivation of cause-effect relationships whilst obscuring the complexity of a given domain making it the most suitable technique to be utilized to fulfill the fundamental purpose of this study. Horn`y [6] shows that Bayesian Networks is the most appropriate choice out of graphical models for dealing not only with uncertainty, but also with complexity and moreover causality. This study will hence contribute to the body of knowledge on stock market anomalies of the CSE and thus showing an avenue to new and existing investors to understand the various market anomalies within CSE.

Materials and Methods

The study is carried out with respect to market data corresponding to a 14-year period starting from 2nd January 2007 and ending at 31st December, 2020. The data within the study needs to be transformed to create variables to test the presence of various selected Calendar Anomalies. A total of seven such anomalies are being modeled with respect to CSE, namely; Day-of-the-Week (DOW) effect [7][13], Month-of-the-Year (MOY) effect [2][3][11], Turn-of-the-Month (TOM) effect [9][12], Turn-of the-Year (TOY) effect [13], Holiday effect [1], Effect of Election days [8] and additionally, Black Friday (BF) effect. The dynamic concept of efficiency has been discussed across literature depicting that inefficient markets can become efficient across time. Hence it is crucial to test the current existence or non-existence of weak form of efficiency within the CSE. Additionally, the stock return which is a continuous variable is discretized into a categorical variable with the aid of the results of Capital Asset Pricing Model (CAPM) and Bayesian

regression. CAPM describes the relationship between the expected return and risk of investing in a security. Accordingly, the expected return of any asset can be expressed as a linear function of the risk-free return and risk premium.
Following the data transformation process and aforementioned analysis, a Bayesian Network algorithm is used to obtain the respective network of the data. R-programming language has been used for the process of fitting the Bayesian Network. The bnlearn package (version 4.7) of the software supports various learning algorithms of which the PC Stable algorithm is utilized within the study.

Results and Discussion

The Bayesian Network resulting from the analysis is as shown in Figure 1 below. Accordingly, a directed edge from a node of a variable to the node of abnormal market returns indicates that there is a causal relationship between the given variable and abnormal market returns. Hence the below Bayesian Network substantiates evidence towards the existence of a causal relationship between abnormal market returns and the Day-of-the-Week and Turn-of-the-Month. This provides empirical evidence that based on the type of day a given trade occurs there is an associated probability to earn abnormal returns.

Additionally, conditional probability distribution is defined with respect to each node of a Bayesian Network which is shown through the resulting Conditional Probability Table (CPT) (Figure 2). CPT exhibits the probabilities of earning each type of return, namely, Normal returns, Abnormal Positive Returns and Abnormal Negative Returns on a given trading day. This enables investors to strategically plan and time their trade and hence maximize their returns. CPT shows a much noteworthy observation whereby the probability of having negative abnormal returns given that the trading day is a Wednesday and first day of the month is as high as 41%.

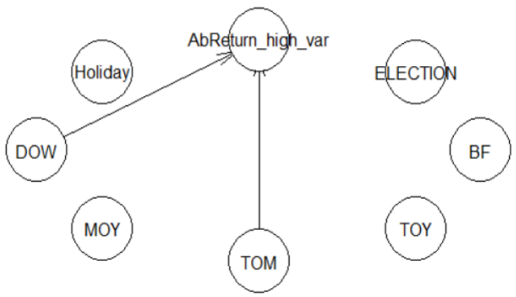

Figure 1. Bayesian Network of Stock Returns of CSE

			Day of week				
			Monday	Tuesday	Wednesday	Thursday	Friday
Turn of Month	First day	Normal	0.513	0.586	0.333	0.421	0.683
		Abnormal Positive	0.210	0.189	0.252	0.271	0.158
		Abnormal Negative	0.277	0.225	0.414	0.308	0.158
	3 days before	Normal	0.741	0.790	0.785	0.607	0.749
		Abnormal Positive	0.129	0.105	0.093	0.228	0.096
		Abnormal Negative	0.129	0.105	0.122	0.165	0.155
	1 week before	Normal	0.779	0.790	0.711	0.711	0.733
		Abnormal Positive	0.125	0.105	0.129	0.099	0.102
		Abnormal Negative	0.096	0.105	0.160	0.190	0.165
	3 days after	Normal	0.575	0.541	0.544	0.612	0.648
		Abnormal Positive	0.196	0.244	0.228	0.179	0.109
		Abnormal Negative	0.228	0.215	0.228	0.208	0.243
	1 week after	Normal	0.648	0.725	0.711	0.680	0.772
		Abnormal Positive	0.176	0.170	0.129	0.160	0.099
		Abnormal Negative	0.176	0.105	0.160	0.160	0.129
	Others	Normal	0.841	0.838	0.804	0.793	0.898
		Abnormal Positive	0.076	0.087	0.107	0.110	0.050
		Abnormal Negative	0.083	0.075	0.088	0.097	0.052

Figure 2. Conditional Probability Table

Conclusions and Recommendations

This study explores the application of Bayesian Networks to establish a probabilistic causal relationship between stock market anomalies and calendar effects with respect to the Colombo Stock Exchange. Following the theoretical literature, empirical studies on the Weak Form efficient market hypothesis and related Calendar Anomalies have been intensively investigated. Empirical evidence proves that the CSE is inefficient and various seasonality within stock returns have been identified. However, the studies fail to establish a causal probabilistic relationship between the identified seasonality and stock market returns. This study is hence carried out with respect to CSE market data corresponding to a 14-year period starting from 2nd January 2007 and ending on 31st December 2020 in order to model such a probabilistic relationship. Accordingly, the built Bayesian Network provides empirical evidence towards the existence of a causal relationship between Day-of-the-Week and Abnormal returns and Turn-of-the-Month and Abnormal returns.

A significant finding of the study shows that probability of having negative abnormal returns given that the trading day is a Wednesday and first day of the month is as high as 41%. This indicates that investors are prone to make very bad investment decisions during this type of days. Hastings and Washington [5] shows that people have a more tendency to spend on instantaneous consumption during the beginning of the month. Furthermore, Miura [10] discusses how two professors from the University of Vermont, Peter Dodds and Christopher Danforth have found that people are most unhappy and stressed on Wednesday by analyzing collective happiness. Hence, it can be seen that the combination of instantaneous spending with pure stress and exhaustion has led to bad investment decisions.

"After two days of the daily grind, my memories of the weekend gradually fade, and my sudden realization of it's still Wednesday, brings on more exhaustion."
-Miura [10]

Accordingly, from a stock seller's perspective, this is the worst time to sell shares since negative abnormal returns indicates that shares are being sold at a loss. However, from a stock buyer's perspective, this is the best time to buy shares since it is being traded in the market at a low price. Furthermore, the resulting CPT enables investors to compare the probabilities of abnormal positive returns and abnormal negative returns to choose the better option of either selling or buying stock on a given trading day. Whereby, a seller may choose to sell on a trading day with comparatively higher probability of abnormal positive returns and a buyer may choose to buy on a trading day with comparatively higher probability of abnormal negative returns thus to maximize their returns.

Hence it can be seen that Bayesian Networks are able to capture causality even within the most complex of domain and through their intuitively appealing interface, it enables effective communication between statisticians and non-statisticians. The main limitation of Bayesian networks is that it requires prior probability; and given that the prior is chosen inaccurately, results may be misleading. Within the study, the Bayesian Network was completely learned through an algorithm, however, given the presence of accurate expert knowledge, the results can be further improved. The discrete form of Bayesian Networks was used in the study which may result in loss of information. Hence, the model can be further developed utilizing techniques corresponding to continuous variable.

The study was carried out using data corresponding to a conventional time period and hence it cannot be directly generalized to an unconventional situation causing economic instability within the country. The said causes may be unreasonable political instability, hyper-inflation, currency crisis and many more factors related to an economic crisis. The model can however be further improved to account for the aforementioned crisis situations making it possible to derive more timely inferences. The probabilities derived through the Conditional Probability Table of this study can accordingly be used as the prior probabilities for such a model and other external factor that might impact the behavior stock returns in such a crisis can additionally be considered based on expert judgment. The derived model is merely the beginning, there are multiple approaches to further improving the model to account for numerous possibilities.

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E-SCHOOL SL: A MOBILE BASE E-LEARNING SYSTEM

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Introduction

The outbreak of novel coronavirus Disease-2019 (COVID-19) put tremendous pressure on a myriad of sectors in the world, whereas the education system lies on top of many others. For instance, by mid-April 2020, well over 1.58 billion learners (pre-primary to higher education) worldwide were severely affected by the pandemic, representing 200 countries [1]. Long closure of the school with obstacles to travel led to a plethora of negative impacts on children as they struggle to continue their education while preventing the disease [2]. In order to address this issue, UNESCO has emphasized the importance of distance learning practices [3], where information and communications technology (ICT) plays a key role. Such ICT-based practices are not new concepts to many developed countries, converse in developing countries. Countries like Sri Lanka, need to be carried out considerable work in this arena, as it has recognized the importance of ICT-based practices as never before [4]. In this aspect, the rapid development of mobile computing devices like smartphones has had a significant impact on a variety of fields, including education. It is interesting to note that, with 53% of the global smartphone market, Android is the most popular operating system [5].

Thus, in this study, the authors attempted to develop a trilingual android mobile application named 'E-SCHOOL SL' (version 1.0), which helps schoolchildren to continue their studies more effectively and also as a much easier approach. The developed android application provides extra practice in addition to distance learning activities. This application was aimed to support the schoolchildren (junior and senior secondary), by giving free and easy access (and also download) to the relevant authorized textbooks (for all subjects), model questions, and past papers with marking schemes. In addition, several other activities (e.g., simulation, statistical analyzer) are also included to enhance the education process.

Materials and Methods

Collection of background data

First, secondary information was acquired by using web search engines including NCBI, Google Scholar, and common Google platform in order to better comprehend the research gaps and the existing state of affairs. Second, the necessary primary data was gathered via an in-depth open-ended questionnaire interviewing 30 school students in different age groups. Finally, individual in-

depth discussions were conducted with several subject experts for further understanding of the requirement.

Development of software

Model-View-ViewModel (MVVM) system architecture was used to construct the "App—E-School SL". The outlook of the system architecture indicates in figure 1. The elements in the layout were built using a hierarchy of View and ViewGroup objects. In this application, layout XML files were used to define the actual UI (User interface).

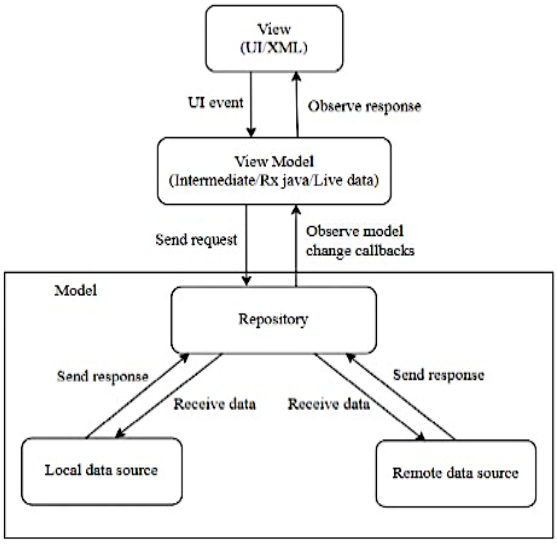

Figure.1. The system architecture

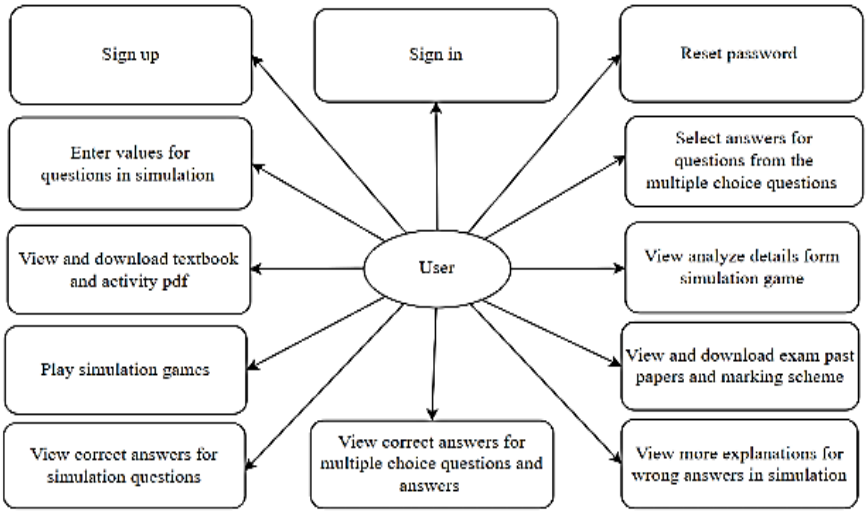

Figure 2. The system overview for user

Results and Discussion

Acceptance testing was carried out with 50 Sinhala 40 Tamil and 20 English language students to check the effectiveness of the system. Overall, the test shows 87% of average acceptance, consequently passed the user acceptance testing. The app provides more features over the handful of currently available apps under this category (e.g., Vibhawa Online Papers, School Text Books in Sri Lanka, SL Education for GCE A/L), in Sri Lanka. For instance, some other available apps are not along with a trilingual facility (Sinhala, Tamil, and English), also restricted to providing only textbooks. This developed app has facilities to read and download school textbooks, model exam papers and past papers with answers in all three languages. Furthermore, the students can get a self-evaluation about the knowledge they gather through the analysis part of this application. The simulation activity page is created for selected subjects like maths and science, which is helpful to train the knowledge gained from the classes. Further, these simulators help the students to easily understand the concepts of aforesaid subjects. Moreover, users can see the scores or analysis for those stimulation queries that they have answered and can assess themselves, thus can progress further using the app.

Figure 3 shows some snapshots of the app. The app also improved with several educational games, which could help to stimulate the students towards the study process. This would be the most recognized feature of this app and no unnecessary pop-ups or bugs visible in this system. In this system, the admin only allows changes in the app, such as the addition or removal of the currently available documents, questions, etc.

A B C D E F G

Figure 3. Snapshots of selected pages. A). Welcome page B). Sign up page, C). English menu page shows the grades of studies to select, D). Activity menu, E) Analysis of performance, F). Simulation marks page and G). Simulation game page

Conclusions and Recommendations

The developed system can be utilized as a user-friendly platform to support the student's education process, with many more supportive tools in a single app. This would also be helpful to stimulate students toward self-learning intension. Therefore, this application would be a more useful tool to keep in touch with the studies if any situations like COVID-19 happen in the future. In the second phase of this study, it is expected to be enhanced this app with recorded video or audio study materials as well.

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FULL PAPERS

FOCUS AREA

Basic Sciences, Emerging Technologies & Indigenous Knowledge

DEVELOPMENT OF A SMALL-SCALE REVERBERATION CHAMBER AND VALIDATION OF THE DIFFUSE FIELD

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Abstract: Noise absorbers are in a growing demand in the automotive, aerospace and building construction industries. Testing acoustic properties of materials is mandatory for developing an efficient acoustic-controlling product design. Two main methods available to measure acoustic properties of materials are impedance tube method and reverberation room method. Impedance tube method uses small test specimens (usually less than 10 cm in diameter) and measures only normal incidence sound absorption, whereas reverberation room method is a relatively expensive setup which usually requires a large space (100-200 m^3) and allows large samples (10-12 m^2). To overcome the drawbacks of the current test methods and to make a comparative analysis of the test samples, a small-scale reverberation room was designed. A chamber with 2.06 m^3 volume was constructed. All the inner surfaces including the door were lined with highly reflective ceramic tiles to obtain maximum reflectivity. The randomness of the incident angles was achieved by an asymmetric shaped room with an inclined roof to obtain all walls non-parallel. A modal analysis was performed to validate the small-scale reverberation chamber for acoustic measurements. The pressure variations inside the enclosure within resonance frequencies are too small. Even though the cut-off frequency of a purely rectangular chamber with the same volume is 270 Hz, the new design of the reverberation room allows taking measurements below 270 Hz.

Keywords: noise, acoustic, reverberation room, cut-off frequency

I. INTRODUCTION

Unwanted sound waves are called as noise and noise pollution causes adverse health effects like cardiovascular diseases, anti-social behavior, increased aggression, hypertension, tinnitus and vasoconstriction [1], [2].

Furthermore, long term exposure to a noise level higher than 85 dB leads to permanent hearing loss [3], [4]. Therefore, room acoustics deals with reducing noise levels and improving the quality of sound within an enclosed space such as a workstation, lecture hall, office,

theatre, recording studio or a concert hall.

Methods of acoustic control can be broadly classified in to two groups as active control and passive control [3]. While passive mediums dissipate acoustic energy through various damping mechanisms, active mediums need the application of external energy in the noise reducing process by destructive interference. Due to complex and expensive electro-acoustic and electronic design, active acoustic controllers are used for low frequency sound control in very limited applications, for example in headsets for helicopter pilots [5]. In room acoustics passive sound absorbers made from porous materials or resonant structures are applied in room space or on the boundaries to reduce sound pressure level or to adjust reverberation time [3]. To develop an efficient passive sound absorber, the knowledge of sound absorption coefficients of materials is mandatory [6].

The two main concepts practiced in laboratory setups for measuring the sound absorption coefficient of materials are the impedance tube method and the reverberation room method [7]. Impedance tube method uses very small test specimens (usually less than 10 cm in diameter) and measures only normal incidence sound absorption, whereas the reverberation room method is an expensive setup which requires a large space (> 125 m^3) and large samples (> 5.5 m^2). To overcome the drawbacks of the current test methods and to make a comparative analysis of the test samples, a small-scale reverberation room was designed and developed. Even though several researchers have developed small scale reverberation chambers with different dimensions, they haven't analyzed the modal behavior inside the room to validate for spatial distribution of the sound field [8],[9],[10],[11],[12],[13]. Therefore, this work is aimed at validating the proposed design using a computational approach.

II. LITERATURE REVIEW

The reverberation room method is an indirect method of measuring random incidence sound absorption coefficient of samples using reverberation time. European regulation ISO 354 and its American equivalent ASTM C432-09 have described the methodology of reverberation room method in detail [14]. A band of random noise is used as a test signal and when the signal is turned off, the reverberation time is measured for each frequency band, before and after placing the test specimen. From the two reverberation times, sound absorptions of each two cases are calculated using a formula (Eq. 1) called as Sabine's formula which was found by Wallace Clement Sabine in 1898 [15].

$$A = \frac{0.16\,V}{T_{60}} \qquad \text{.... (1)}$$

Where, A is the total absorption of the specimen in Sabins, V is the volume of the reverberation room in m^3 and T_{60} is the reverberation time in seconds. The difference of two absorptions divided by sample area is attributed to the sound absorption coefficient of the test sample α,

$$\alpha = \frac{A_2 - A_1}{S} \qquad \text{.... (2)}$$

Where, A_1 is the absorption of the empty reverberation chamber, A_2 is the absorption of the reverberation room after the specimen has been installed and S is the area of the test specimen in m^2.

Sabine's formula is derived based on the following two assumptions.

1. Incident sound field is diffuse before and during decay.
2. No additional energy enters the room during decay [16].

Therefore, the sound field within the reverberation room should fulfill these assumptions to utilize Sabine's formula. The proper functionality of a reverberation room relies on the proper dimensions and the shape of the room. An acoustic field is considered to be perfectly diffuse when sound energy is uniformly distributed at every point and the angle of incidence is random [17]. In order to use Sabine's formula, the sound field inside the room should be diffuse and Sabine's formulation should be applied when the mean absorption within the room is less than 0.4 [7]. Furthermore, changes in temperature and relative humidity during the measurement period may have a large effect on the reverberation time. The average temperature shall not be less than 10 ℃ and deviations from the average temperature of the room shall not exceed 5 ℃. The average relative humidity in the room shall not be less than 40% and deviations from the average relative humidity shall not exceed 5% in the measured relative humidity [18].

The Reverberation room method is preferable for acoustic measurements as sound absorption coefficients of any type of absorber can be tested in realistic conditions. But, for research purposes, it is not economically viable to build such large rooms and large samples with a sample area of at least 10 m^2. So, several researchers have proven that small scale reverberation chambers are adequate for qualitatively distinguish the acoustical behavior of structural panels with repeatable and reproducible measurements [8],[9],[10],[11],[12],[13].

However, achieving a diffuse sound field within a small-scale reverberation room at low frequencies is complicated. When sound waves are reproduced in an enclosed space the room dimensions encourage certain frequencies for

resonance behaviors. These resonances are known as room modes or standing waves which introduce irregularities in the frequency domain response of a room by modifying the pressure values of specific points in the chamber. In small spaces, the number of room modes per unit volume (modal density) for low frequencies is low and it results in individual modes being clearly audible. But in mid and high frequencies the modal density is so high and the adjacent modes sum together to give a broad frequency independent response [19]. Therefore, the volume of the chamber (V) determines the cutoff frequency which room modes start to cluster very closely together that they are no longer seen as resonant peaks; this point is known as the Schroeder frequency (F_c) and calculated using the equation 3 [20].

$$F_c = \frac{C}{\sqrt[3]{V}} \quad \text{.... (3)}$$

Where C is the speed of sound in air. At higher frequencies, number of room resonance modes increases rapidly and tends to fill both the frequency spectrum and angle of incidence uniformly [21]. But in low frequencies poor diffusion happens due to the low number of possible resonance modes. In order to obtain an adequate diffusion, the least number of permissible room modes in the measurement bandwidth should be 20 according to previous research [22].

The most straightforward way of achieving a diffuse sound field is using large rooms with a volume of at least 100 m^3 whose walls are uniform, smooth, and rigid as possible. But there are some drawbacks when using large rooms like high cost and increase of contribution of air absorption to the total room absorption violating Sabine's necessary assumption of negligible energy loss during a mean free path transit for the validity of the reverberation equation. Therefore, this study is focused on developing a small-scale reverberation room with the use of special techniques such as using non-parallel walls made out from special materials to achieve a diffuse field.

III. MATERIALS AND METHODS

A small-scale reverberation chamber was designed according to the dimensions shown in figure 1. The volume of the reverberation room is 2.06 m^3. The outer walls were made out from 15 mm plywood to comply with weight constraints. Steel box bar frames were used to strengthen the structure. All the inner surfaces including the door were lined with highly reflective ceramic tiles to obtain maximum reflectivity. Ceramic tiles ensure repeated reflections and increase the time of sound decay. The randomness of the incident angles was achieved by an asymmetric-shaped room with an

inclined roof to obtain all walls non-parallel.

The positions of the sound source, microphone and test specimen were determined according to the specifications mentioned in standards. A speaker was placed facing into trihedral corners of the room to achieve adequate diffusion. microphone with switchable polar patterns was used to measure reverberation times inside the chamber. Omni directional polar pattern and flat random incidence amplitude response were used over the range of frequencies.

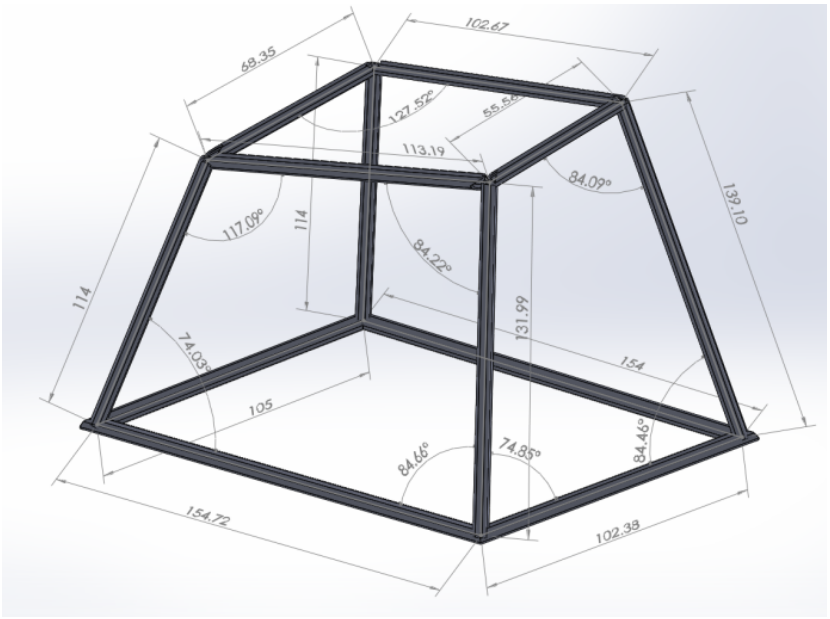

Figure 1. Dimensions of the reverberation chamber

A band of random noise with a continuous spectrum covering the frequency range of the measurements from 100 Hz to 10000 Hz was used as test signal. Test signal generation and reverberation time measurements were made with a one third octave band real-time analyzer, 'Room EQ Wizard software'. AT-2050

The axial, tangential and oblique room modes (eigenmodes) for rectangular rooms can be calculated using equation 4 [23].

$$f = \frac{c}{2}\sqrt{\left(\frac{n_x}{L}\right)^2 + \left(\frac{n_y}{B}\right)^2 + \left(\frac{n_z}{H}\right)^2} \quad \text{.... (4)}$$

f = Frequency of the mode in Hz
c = Speed of sound (343 m/s at 20 °C)
n_x = Order of the mode of the room length
n_y = Order of the mode of the room width
n_z = Order of the mode of the room height
L, B, H = Length, width, and height of the room in meters [24].

For rooms of more irregular shapes, it is not as easy to compute the resonances as it is for a rectangular chamber of rigid boundaries. However, using computational techniques such as finite element method, the resonance frequencies and eigenfunctions of nonrectangular rooms can be determined numerically.

To validate the required diffuse field inside the empty reverberation chamber, a finite element model shown in figure 2 was developed and simulated using COMSOL Multiphysics software. The finite element mesh consists of 1316 tetrahedral elements. All walls were defined as acoustically hard.

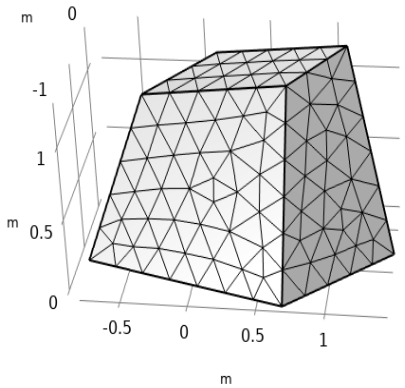

Figure 2. The finite element mesh of the reverberation room

IV. RESULTS AND DISCUSSION

Modelling sound pressure distribution within the reverberation chamber is important to test the accuracy of the experimental results. The highest-pressure variations within an enclosure happen in natural resonance frequencies which are called as eigen frequencies. Therefore, the modal analysis must be performed to validate the small-scale reverberation chamber for acoustic measurements. Three-dimensional model with same dimensions and materials discussed earlier was built. A wide range of equations were used to specify subdomains, boundaries, edges and points including Givoli and Neta's reformulation of the Higdon conditions for plane waves [25]. The theories behind this model are based on basic equations of fluid dynamics.

Figure 3. Developed small scale reverberation room

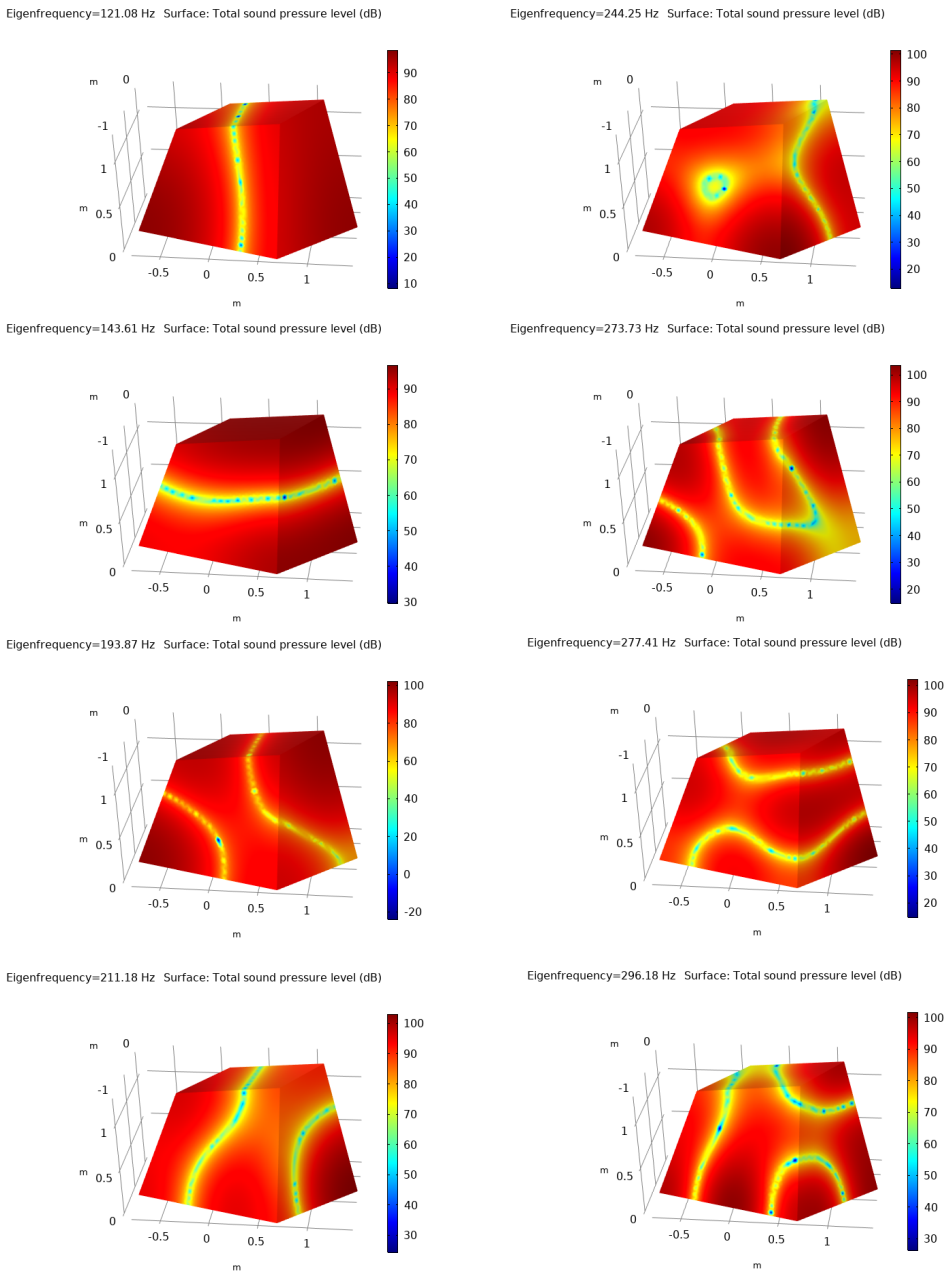

Figure 4. Acoustic eigen modes 1 to 8. A): mode 1, 121.08 Hz, B): mode 2, 143.61 Hz, C): mode 3, 193.87 Hz, D): mode 4, 211.18 Hz, E): mode 5, 244.25 Hz, F): mode 6, 273.73 Hz, G): mode 7, 277.41 Hz, H): mode 8, 296.18 Hz.

Using the eigenfrequency analysis, the pressure variations inside the chamber during first 8 eigenmodes are depicted in figure 4. The lowest eigenfrequency of the room is 143.61 Hz. The pressure variations inside the enclosure during resonance frequencies are too small. Therefore, we can conclude that, even though the theoretical cut-off frequency of the room is approximately 270 Hz, the new design of the reverberation room allows taking measurements below 270 Hz.

The reason is, in rooms of more arbitrary shape, a more randomized distribution will automatically be obtained.

V. CONCLUSIONS

The results of the simulated model indicate the superiority of the small-scale reverberation chamber to the regular large reverberation room, concerning the distribution of resonance frequencies and the spatial distribution of the sound field. By developing a small-scale reverberation chamber using the dimensions and materials mentioned in this paper, scientists, researchers, or sound engineers can accurately measure acoustic properties and calibrate acoustical instruments.

ACKNOWLEDGEMENT

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SYNTHESIS OF GRAPHITE OXIDE AND EFFECT OF ITS LOADING ON CURE CHARACTERISTICS AND AGEING PROPERTIES OF NATURAL RUBBER COMPOSITES

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Abstract: Graphite derivatives are playing a great role in the world in every aspect. Among these, graphite oxide (GO) is one of the recent miracle materials as it has many applications in different fields**.** In this study, GO was synthesized by improved Hammers' method and it was characterized via fourier transform infrared spectroscopy (FTIR), X-ray diffraction spectroscopy (XRD), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM). Subsequently, natural rubber (NR) composites were prepared by varying the GO loading from 0 phr (parts per hundred rubber) to 10 phr at 2 phr intervals. Maximum torque and delta cure results indicated high cross-link density for the composites prepared with GO. In contrast, the stock viscosity of the composites decreased with the increase of GO loading. In addition, the composite prepared with 10 phr loading of GO showed the highest delta cure (M_H-M_L) indicating the highest hardness. Further, the composite prepared with 8 phr loading of GO showed the highest scorch time indicating the highest processing safety. However, cure time slightly increased with the increase of GO loading and, slower cure rate was indicated at higher GO loading. Furthermore, thermal stability of the GO / NR composites prepared with 8 and 10 phr loadings of GO was at a high level. In overall, the composite prepared with 10 phr loading of GO which exhibited better cure characteristics and thermal stability compared to the others would be suitable for particular dry rubber-based applications.

Keywords: Graphite oxide, natural rubber, minimum torque, cure time, ageing properties

I. INTRODUCTION

Synthesis of graphite oxide (GO) is carried out by placing graphite in concentrated acid in the presence of an oxidizing agent. The improved Hammers' method demonstrated a less hazardous and more efficient method for graphite oxidation. Further, GO is a product obtained from oxidation of graphite, which maintains the original layered structure of graphite. This is because

of the existence of large amounts of hydroxyl (-OH), carboxylic acid (-COOH), carbonyl (-C=O), epoxide functional groups (C-O-C) and other oxygen based functional groups attached onto the surface or edge plane [1].

These oxygenated groups are hihly responsible for many advantages over graphite, due to higher solubility and the possibility for surface functionalization which have offered several opportunities for use in nanocomposite materials [2].In addition, graphite derivatives have proven to be active fillers in polymer composites recognitions their ideal material properties and dispersibility in polymer matrice [3] Moreover, GO has increasingly attracted attention owing to its fascinating physico-mechanical properties including thermal and electrical properties. These unique properties hold great promise for potential applications in many technological fields such as electronics, packaging material, sensors, batteries, capacitors, hydrogen storage, etc [3,4]. The main aim of this study is to synthesize GO by the improved Hammers' method [5], that is, graphite, as a beginning material evaluating the effect of different processing parameters such as concentration, temperature, and reaction time to prepare graphite oxide successfully [5]. This was mainly carried out using sulfuric acid, phosphoric acid, and potassium permanganate excluding sodium nitrate which emits poisonous gases into the environment. Not only that, graphite-based fillers especially GO, reducible graphite oxide (rGO), graphene, etc. have focused global attention in the industry and academia due to their massive properties such as physical properties, high electron mobility, thermal conductivity, mechanical stiffness, strength, and elasticity.

In addition, literature reported that physico-mechanical properties enhance graphite oxide from waste batteries and maize cob carbon as filler in rubber compounds [6]. However, Carbon black is widely used as filler for rubber in the industry due to carbon black in rubber could introduce massive changes in the mechanical, thermal and physical properties [6]. However, a higher loading of carbon black should be incorporated to obtain the required properties of end rubber-based products. Higher loading of carbon black causes environmental pollution and poor dispersion in the polymer matrix and further, agglomeration in the polymer matrix. Therefore, in this study, lower loading of GO was used to enhance the performance of NR composites, and further, it would be advantageous for volumless rubber-based applications.

II. METHODOLOGY

Materials

RSS-2 (smoked rubber sheet) consisting of a plasticity retention index of 64 was supplied by the Rubber Research Institute of Sri

Lanka. Graphite having a mean particle size of 14 micron was used as a filler and was obtained from Bogala Graphite Lanka PLC., Sri Lanka. All rubber compounding ingredients were purchased from local suppliers. Potassium permanganate ($KMnO_4$), ethanol (C_2H_5OH), hydrochloric acid (HCl), sulfuric acid (98% H_2SO_4), ($NaNO_3$)sodium nitrate and hydrogen peroxide (H_2O_2) were purchased from Organic Trading (Pvt) Ltd, Sri Lanka.

Synthesis of Permanganate-GO

GO was synthesized by the Improved Hammers' method. In this method, 15 g of graphite was mixed with 45 g of $KMnO_4$, and diluted slowly in 400 ml of sulfuric acid. The solution was stirred for 3 h at 50 ^oC. The oxidation was stopped by adding a mixture of 3 ml of H_2O_2 and 400 g of flake ice, and the solid was filtered off under vacuum. The mixture was washed with 200 ml of distilled water and HCl. The resulting filter cake was dried at 60 ^oC during 6-12 h. The product was denoted as Permanganate-GO.

Preparation of NR composites filled with GO

A series of NR composites was formulated by varying the GO loading from 0 to 10 phr at 2 phr intervals. The NR composite prepared without GO was considered as the control. The formulation of the composites is given in Table 1. The composites were prepared by melt mixing using a Brabender Plasticorder operated at room temperature, at a rotor speed of 60 rpm. Total mixing time was kept constant at 10 min. Mixing cycle used in the preparation of NR composites is given in Table 2. The composites were compressed in an electrically heated hydraulic press machine at 150 ^oC under a pressure of 0.35 MPa to produce 2 mm thick sheets. Test specimens were cut from these sheets according to the standards.

Table 1. Formulation of NR composites filled with GO

Ingredient	**Phr**
NR	100
ZnO	5.0
Stearic acid	2.0
TMQ	1.0
GO	0 2 4 6 8 10
ZDC	1.5
Sulphur	2.0

ZnO - Zinc oxide
TMQ - 2,2,4-trimethyl-1,2-dihydroquinoline
ZDC - Zinc diethyldithiocarbamate

Table 2. Mixing cycle of the GO / NR composites

Total time, min	**Ingredient**
0	Added NR
1	Added zinc oxide + stearic acid + TMQ
2	Added GO
6	Added ZDC
8	Added sulphur
10	Dumped the compound

Characterization

X-ray diffraction (XRD) analysis was conducted using X-ray diffractometer (Ultima IV, Japan) to confirm the crystallographic nature of GO material. The GO was scanned in continuous mode by varying the scanning angle from 0.50 to 90.0 degrees at a scanning speed of 3 degrees per minute in 2θ (degrees). Further, chemical structures of GO were characterized using the Nicolet 380 FTIR spectrometer Spectra were recorded in the range of 400 –3500 cm^{-1} operated at 4 cm^{-1} resolution. Surface morphology of GO was examined by Scanning Electron Microscopy (SEM) using a ZEISS EVO LS 15 Microscope. Further, thermal stability of the GO investigated through thermogravimetric analysis (TGA) (TGA 400, Perkin Elmer) at a heating rate of 10 ˚C / min from room temperature to 600˚C in a nitrogen atmosphere.

Cure characteristics

Cure characteristics of GO/NR composites, such as minimum torque (ML), maximum torque (MH), scorch time (Ts_2), optimum cure time (T_{90}), cure rate index (CRI), and extent of cure or delta cure (MH-ML), were obtained by a Dynamic Rubber Process Analyzer (D-RPA 3000- MonTech, Germany) at 150 ˚C.

Ageing properties

Accelerated ageing of the composites was carried out at 70 ˚C for 72 hours in an air circulating oven. Aged tensile properties were evaluated using Instron tensile testing machine according to BS ISO 37:2017 and percentage retention of these properties were calculated according to Equation 1.

$$\text{Retention of tensile strength \%} = \frac{\text{Tensile strength after ageing}}{\text{Tensile strength before ageing}} \times 100 \quad \ldots. 1$$

III. RESULTS AND DISCUSSION

Characterization of GO

FTIR spectroscopy is a technique for studying the movement of chemical bonds by measuring the absorption of electromagnetic radiation from a compound. This analysis is based on the excitation of molecular bonds in a sample by infrared radiation of frequencies between 4000 and 800 cm^{-1}. It is a useful tool for the rapid characterization of GO. The FTIR signals are interpreted as hydroxyl (OH), ketone (C=O), C-O, C-H, etc. Further, the FTIR spectrum of GO is shown in Figure 1. The most significant transmittance bands in the spectra include the stretching and in-plane deformation of O-H bonds in the hydroxyl groups found at 3300 -3500 cm^{-1} and 1300–1500 cm^{-1}, respectively and the C=C ring stretching at 1608 cm^{-1}. Moreover, the two absorption peaks at about 1226 cm^{-1} and 1044 cm^{-1} are attributed to the stretching vibrations of the C-O group. According to this spectrum, number of functional groups of graphite oxide are observed due to the

intercalation of graphite. In addition, results obtained by the FTIR confirmed the existence of oxygen-containing groups on the GO in which the main absorption band at 3340 cm^{-1} is attributed to the stretching vibrations of the O-H group [1].

X-ray diffraction patterns for graphite and GO powders were recorded (Figure 2). The diffraction peak for graphite is at 2ϑ =26.6° with a corresponding layer to layer distance of 0.337 nm, which is similar to the reported value in the literature [7]. After oxidation of graphite, the diffraction peak for GO is at 2ϑ = 9.6°. The patterns show a larger interlayer spacing of GO than graphite layers due to the formation and introduction of oxygen-containing functional groups between layers. These functional groups have facilitated the hydration and exfoliation of GO in water. In addition, the pattern of GO is also not smooth. This might be due to the irregular spacing of GO layers [8].

Thermal reduction of GO is a well-known method for removing oxygen-based components from its surface. In this process, oxygen is removed in the form of H_2O, CO_2 and CO. This GO reduction can be described using TGA. The TGA of GO and graphite are shown in Figure 3. Graphite does not show any significant weight loss up to 500° C. However, the figure shows that GO is thermally unstable and at temperature below 100 ° C, it begins to lose weight. According to the results, GO represents two stages of degradation. Mainly these weight losses occur due to the decomposition of oxygen-containing functional groups. In general, the loss of H_2O and CO_2 due to the removal of the OH and C-O-C active groups during thermal reduction in GO is considered to be the higher weight loss clearly seen in the range 150–200°C. Furthermore, The gradual loss of GO shown in the range 240–500°C can be attributed to the degradation of the left over C–O and C–H moieties.

Surface morphologies of graphite and graphite oxide were studied using SEM. SEM micrograph of the graphite surface (Figure 4) does not show good adhesion between the graphite particles and also, most particles are irregular in shape. However, the micrograph of GO (Figure 4), shows a loose sponge structure as shown in Figure 4 (b).

Also, it can be seen that graphite particles of GO are interconnected and uniformly distributed due to the presence of a large number of functional groups as indicated by the FTIR spectrum (Figure 1). Further, the SEM results show that the morphology of GO appears as a tight layer with a wavy surface which is sometimes wrinkled.

Cure characteristics of GO/ NR composites

Rheographs provide important characteristics namely, scorch time, cure time, minimum torque, maximum torque, delta cure and

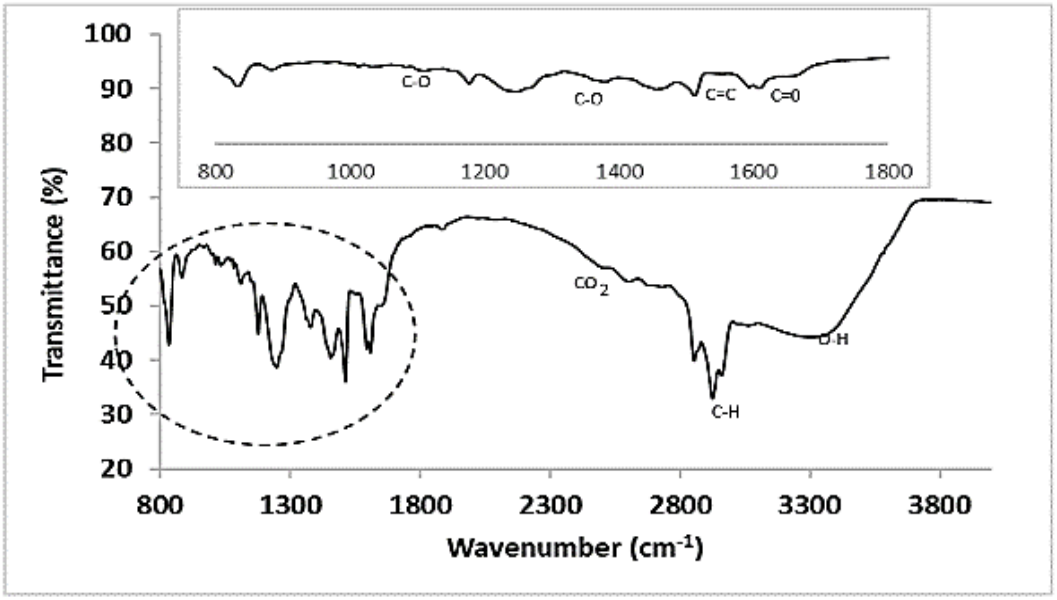

Figure 1. FTIR spectra of GO

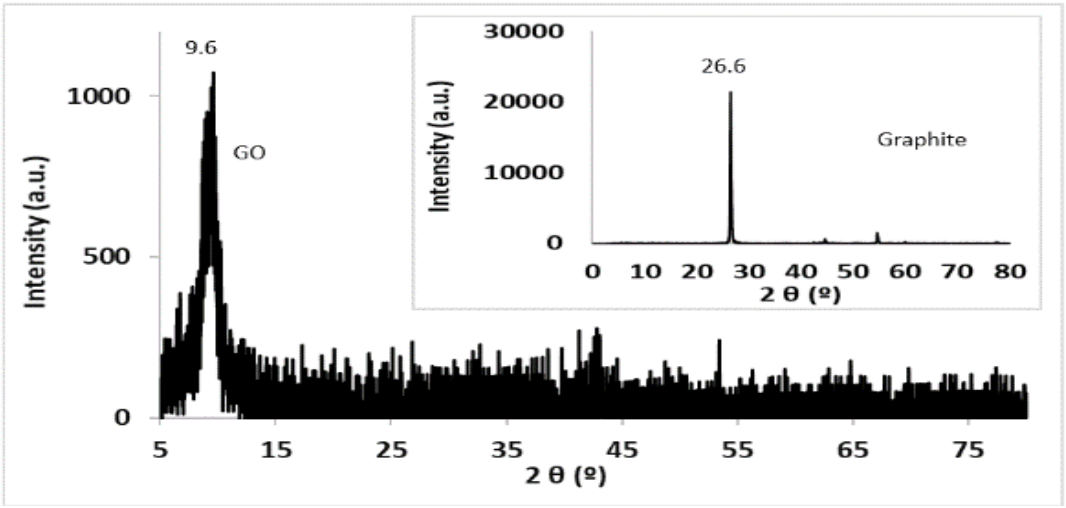

Figure 2. XRD pattern of GO

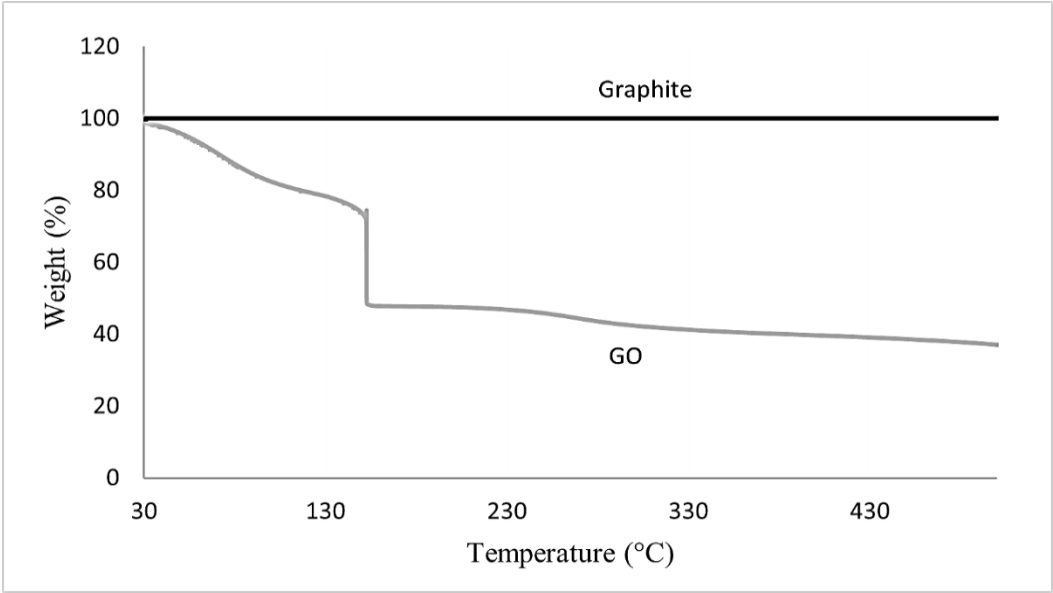

Figure 3. TGA curves of graphite and GO

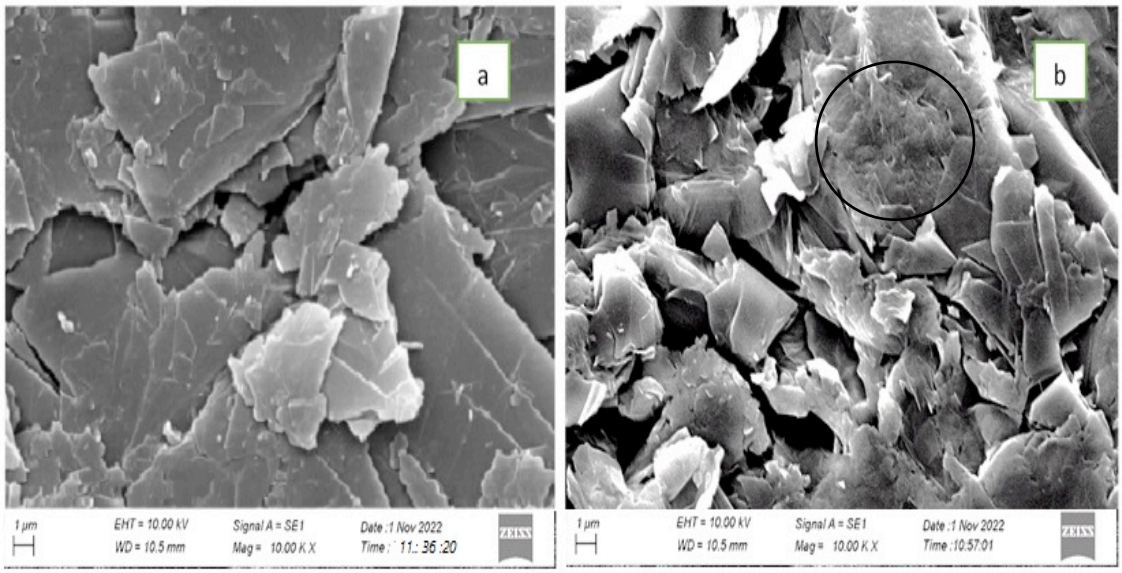

Figure 4. SEM images of surface (a) graphite and (b) graphite oxide

cure rate of a rubber composite. The scorch time (ts_2) is usually defined as the onset of vulcanization of a composite at a specific temperature, which represents the time limit available for processing. Cure time (t_{90}) is the time required for 90% vulcanization of the composite to achieve the required degreeof cross-linking to yield the desired properties. The minimum torque (M_L) is mainly related to the processability and viscosity of the unvulcanized stock. The rheographs of GO/NR composites are shown in Figure 5. According to these curves, the composites prepared with 0 phr and 10 phr GO show similar patterns, but they differ from the curves of the other four composites.

Minimum Torque (M_L)

Minimum torque is an indication of stock viscosity and processability of rubber composites. As shown in Figure 6, the minimum torque values of the composites decrease with the increase of the GO loading. The results obtained show the highest value for the control (without GO) composite and the other composites show significant similarities from 2Phr to 10Phr. The main reasons for this may be wettability and the low density of graphite-based material [9]. Further, lower minimum torque indicates a lower viscosity and it may be due to better dispersibility of filler and low force requirement for the torque generated initially [9]. Hence, the composite prepared with 10 phr loading of GO shows the lowest minimum torque and indicates the lowest viscosity in comparison to the other composites.

Maximum Torque (M_H)

Maximum torque is an indication of the state of cure. Further, maximum torque may give an idea of the maximum extent of curing such as crosslink density and static/ shear modulus or hardness of the fully vulcanized compound. As shown in Figure 7, maximum torque values of the composites increase with the increase of GO and the highest M_H value is exhibited by the composite prepared with 10 phr GO. It can be due to the improved reinforcing efficiency, better dispersion and distribution of filler in the NR composites and greater cross-link density [10]. Other than that, significant variation of M_H values could not be seen between 4 phr and 8 phr loadings of GO. Furthermore, the gradual addition of GO loading facilitates the generation of cross-linkers, which in turn increases the rigidity or toughness of the composite.

Delta Cure (M_H- M_L)

The difference between maximum torque and minimum torque represents the level of crosslink density of a composite [11]. The delta cure values of NR composites are illustrated in Figure 8. Further, as shown in Figure 8, when the loaded amount of GO powder increases, the delta cure value increase compared to the control composite and the

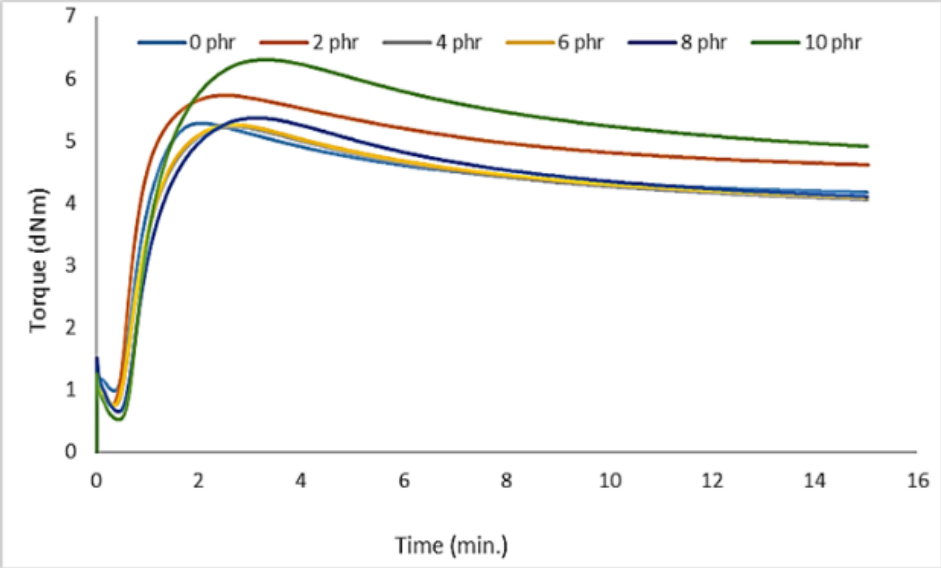

Figure 5. Rheographs of GO/NR composites

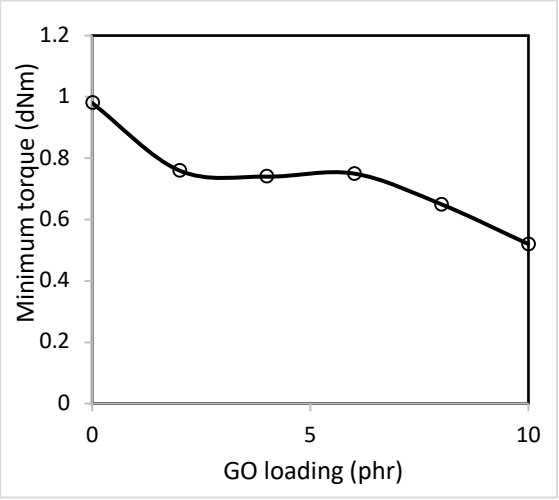

Figure 6. Minimum torque of GO/NR composites

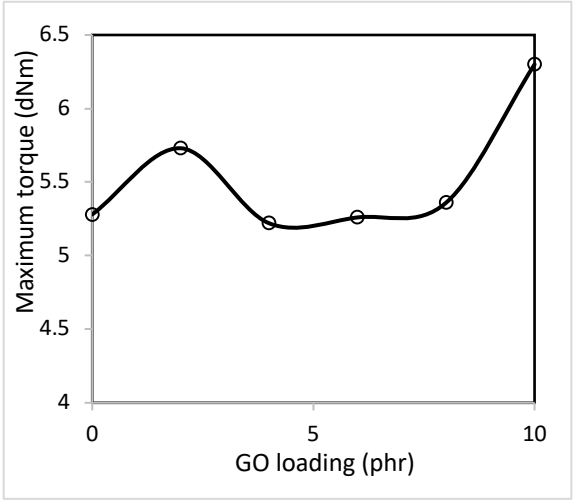

Figure 7. Maximum torque of GO/NR composites

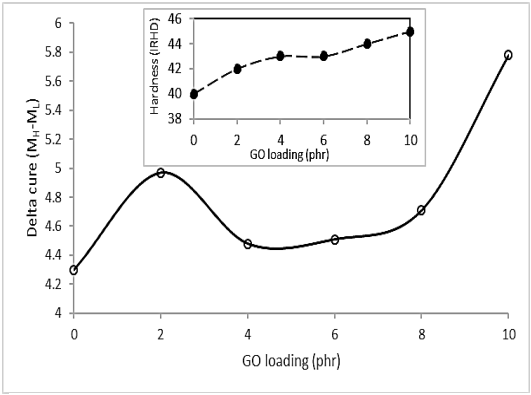

Figure 8. Delta cure of GO/NR composites

highest $M_{H-}M_L$ value is shown by the composite prepared with 10 phr loading of GO. Moreover, the delta cure is generally related to the cross-link density of NR composites. An increase in the value of delta cure indicates an increase in cross-link density. Therefore, the composite prepared with 10 phr loading of GO indicates the highest crosslinking density and hence, the former composite illustrates better hardness properties according to Figure 8. This indicates that the incorporation of filler into the rubber matrix leads to higher viscosity and modulus of rubber composites. Furthermore, similar trend could be observed in maximum torque and delta cure of GO-filled NR composites. Therefore, maximum torque directly correlates to delta cure.

Scorch Time (ts_2)

The variation of scorch time of the composites with GO loading is shown in Figure 9. These values give information about premature cure indicating processing safety. According to Figure 9, the addition of GO from 2 phr to 8 phr shows an increasing trend in the scorch time. This improvement may result as a consequence of the change in filler parameters such as higher surface area, surface reactivity, particle size, and moisture content [9]. When compared with the control composite, all GO-filled NR composites show higher scorch time. Further, composite prepared with 8 phr loading of GO illustrates the highest scorch time and better processing safety. In general, scorch time decreases due to the restriction of mobility and deformability of the matrix with the introduction of mechanical restraints. Therefore, higher filler loading would cause to decrease scorch time of rubber composite. Hence, a slightly reduction is observed in the composites prepared with 10 phr GO compared to 8 phr GO.

Cure Time (t_{90})

The cure time (t_{90}) is the time required for vulcanization to yield the required amount of cross-linking to get the desired properties. Figure 10 shows the 90% cure time of GO/NR composites. According to Figure 10, addition of GO from 0 phr to

10 phr, the cure time is increasing gradually. Most of the time, this improvement is a consequence of filler parameters such as higher surface area, surface reactivity, particle size, and moisture content [12].

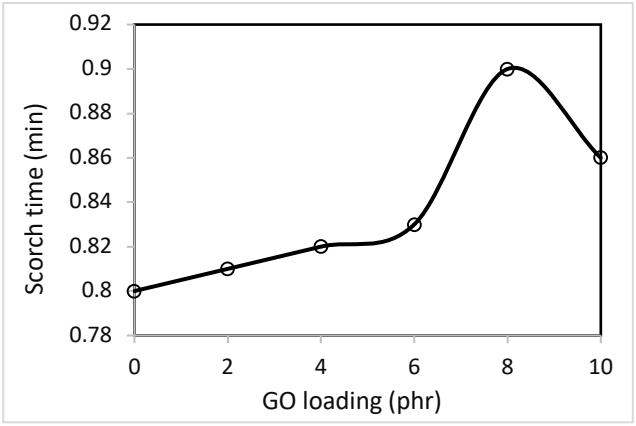

Figure 9. Scorch time of GO/NR composites

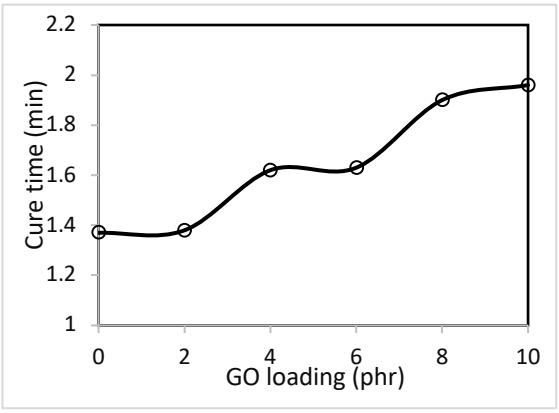

Figure 10. Cure time of GO/NR composites

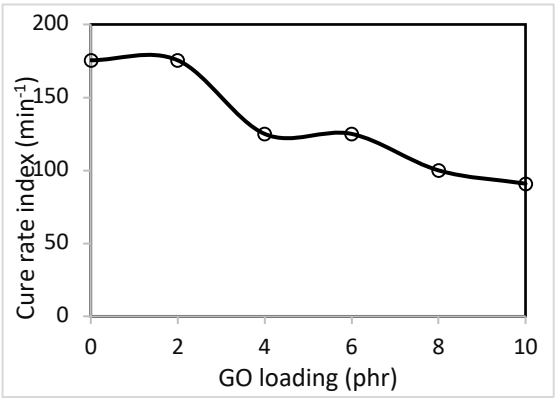

Figure 11. Cure rate index of GO/NR

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Table 3. Retention of tensile properties of GO/NR composites

Property	graphite oxide loading					
	0 Phr	2 Phr	4 Phr	6 Phr	8 Phr	10 Phr
Tensile strength (MPa), after ageing	5.5	3.7	4.7	13.4	22.1	24.1
Retention of tensile strength (%)	21	14	17	55	84	84
Elongation at break (%), after ageing	358	284	350	474	532	555
Retention of elongation at break (%)	68	52	56	76	88	89

However, According to Figure 10, when adding GO powder at 10 phr, shows the highest curing time compared to the other GO filler NR composites and control.

Cure rate index

The Cure Rate Index (CRI) is a measurement of the cure rate of composite based on the difference between the optimal cure time (t_{90}) and the initial scorch time (t_{S2}). According to Figure 11, The CRI values of the GO-filled NR composites are lower than that of the control composite. However, there is no variation in the cure rate of composites prepared with 4 phr and 6 phr loading of GO. A faster cure rate index has been reported for fillers having lower surface area. A moderate increment of the cure time with the loading of graphite indicates a slight increase in production time. In addition, the slight delay in vulcanization at higher filler loadings is a known effect in rubber vulcanization [13]. Hence, the composite prepared with 10 phr loading of GO illustrates the lowest processing rate.

Aged tensile properties of GO/NR composites

Table 3 shows the tensile properties, after ageing of the control, and the other NR composites prepared with different GO loadings. The composites prepared with GO loadings of 8 phr and 10 phr have shown higher tensile properties after ageing and hence exhibits higher resistance to thermal degradation [14]. The control and NR composites prepared with GO loadings of 2 phr and 4 phr indicate poor resistance to thermal degradation. In other words, during ageing, low loadings of GO are not sufficient to retain strong interactions formed with the NR matrix.

IV. CONCLUSION

GO was synthesized successfully using potassium permanganate as oxidizing agent. Transformation of graphite to GO was confirmed by FTIR, XRD and TGA techniques and SEM analysis. The composite prepared with 10 phr loading of GO shows better cure characteristics in terms of maximum torque, delta torque, scorch time, etc. The 10 phr GO filled NR composite showed an increment in scorch time and cure time by 7.5% and 43%, respectively in comparison to the NR composite prepared without GO (control). Further, the composites prepared with GO indicate low energy combustion due to the low cure time exhibited. Hence, results suggest that the reduction of cure time of GO incorporated NR composites would be an advantage for high-energy combustion rubber-based applications. Moreover, ageing resistance of the GO / NR composites prepared with 8 and 10 phr loadings of GO was at a high level. Hence, former composites could be used for high heat resistant rubber applications.

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CRITICAL APPRAISAL OF AYURVEDA PUNSAVANA KARMA

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Abstract: Male sex organs develop in embryos with XY chromosomes, while female sex organs develop in embryos with XX, means the sperm determines a baby's sex! The maternal influence on this is almost unheard. Objective of the review is to find out the maternal influence by Pumsavana Karma on determining the fetal sex. Ayurveda-textbooks, related research articles were critically reviewed and data were gathered. Maternal influence on personality, hormonal statuses, blood pressure, genital status, psychological and behavioral effects of fetus, were described both ayurveda and modern in similar pattern. Maternal Follicular-testosterone levels were found relationship with offspring sex; higher levels are associated with violent/dominant mothers who are having more male offspring. Some herbs are found anabolic, steroid positive and produce testosterone to give birth to son. Women with a male fetus have slightly high TSH levels and a high upper limit of the first-trimester TSH reference range. Females with high-corticosteroids produced more daughters. Nasya, one of the Pumsavana methods stimulates the pituitary glands to regulate hormonal production. Mothers with high systolic blood pressure reported to have high chances of male offspring. During Marsha Nasya, temporary increase in blood pressure is observed. Swimming behavior and selective entry of sperm into genitalia helped explain the possible mechanisms of sexual selection and found significant relationship between fetal male sex and alkaline vaginal pH, and female sex with acidic pH. Pumsavana mentioned, sperm unites ovum in a clean and healthy genital tract is home to an embryo with desired characters. There's greater spontaneous loss of frail male fetuses in response to societal stressors. Pumsavana has a benefic relationship with maternal psychological health and behaviors.
It had being reported Pumsavana Karma can influence the maternal factor on determining the sex of the fetus by several mechanisms. Further, sex determination is highly influenced by the mother.

Keywords: Maternal, offspring, sex determination, Pumsavana Karma

I.INTRODUCTION

Different cultures seem to have the same belief that a mother gives birth to a boy or a girl. In a family where a son is not born, there are instances

where the mother is psychologically oppressed into believing that she is to blame. However, in modern society, this belief has been disproven, and it is now believed that the husband determines whether a daughter or a son will be born. However, in this review, all of the responsibility is placed on the female reproductive system, and the only thing men can do is release sperm into the vagina. The preference for either an X or Y chromosome carrying spermatozoa for fertilization is the result of changes to millions of spermatozoa in the female system, including killing many, lifting, transporting, activating, and permitting for their motility like processes done by female genital tract.

According to the Ayurvedic view; it is possible to give birth to a boy by applying various strategies. The mother has recommended a variety of dietary supplements and medications to be used in this regard. That is, certain changes in the mother's body can change the mother's factors that determine the sex of the baby. The mother's factors determine whether the X chromosome in the ovum is associated with the X chromosome in the sperm or with the Y. This contradicts the widely held belief that the father's male Y chromosome is the sole determinant of the sex of his offspring. Aim of this study was to analyze the Ayurveda Pumsavana karma and find out maternal influence on offspring sex determination.

II. RESEARCH METHODOLOGY

Literature review was conducted in both ayurveda and modern scientific research articles and Ayurveda Samhitha/text books related to sex determination of an embryo. Google scholar, pub med, Medline ware the search engines used with the key word “embryo sex determination”. Articles written in English were considered. Ayurveda text books translated to English and some of the Sanskrit books were referred.

Collected data were compiled and develop an argument on action of Pumsavana karma on sex determination of embryo.

III. RESULTS AND DISCUSSION

In humans, there is evidence that having sons has a higher physiological cost than having daughters. To maximize reproductive success, parents should manipulate the sex of their offspring born in terms of access to physical resources such as food [1].

Pumsavana karma is a process described in Ayurveda for producing desired sex progeny [2]. One of the sixteen rituals which are advised in Ayurveda known as Shodhasha Karmas is Pumsavana. This samskara or treatment can be carried out either before or right after conception. The wise physician acharya Charaka suggests using

genetic engineering to change sex. If mother follows his advice, she will undoubtedly give birth to a boy during her delivery. (Puman Syevyatha Anena Karmana Ithi Pumsavana Karma) the process by which promotes the male embryo is known as Pumsavana. This process is conducted during the first three month of the pregnancy. According to the Ayurvedic view, it is possible to give birth to a boy by applying various strategies. The mother has recommended a variety of dietary supplements and medications to be used in this regard [2].

Understanding the swimming behavior of X and Y chromosome-bearing spermatozoa in the female genital tract, as well as evidence of selective insertion of spermatozoa in cervical mucus, can help to explain the potential mechanisms of sex determination. The factors affecting the motility of sperms in the genital tract are pH, temperature, viscosity, osmolality, and ions. All this evidence suggests that sexual selection depends on the female sexual environment, which changes throughout the cycle, and therefore, by altering the female reproductive system through the use of foods and drugs administered on specific days of intercourse as mentioned in Ayurvedic texts can contribute to the selection of desired progeny.

The concentration of sperm in semen increases if the coitus is performed on alternate days. More the number of Y-bearing spermatozoa, the more probability of procuring a male progeny [3]. The dates indicated for Pumsavana may have a strong influence with this.
Researchers are still interested in finding about the fundamentals of chromosome inheritance, as well as the maternal and paternal factors that influence sex determination. This resulted in a radical shift in our understanding of sex determination, with both parents discovering that they are equally responsible for determining the sex of their offspring. The offspring's gender is determined during the pre-zygotic stage and is determined by natural selection [4]. Pumsavana advised consuming herbs on the same day that a conception is known to have occurred. She should swallow the remedy, combined with the roots of the Lakshmana (Ipomoea sepiaria ROXB), Vata (Ficus bengalensis Linn), Sahadevi (Vernonia Cinerea), Vivadeva (Sida veronicaefolia Lam.), without spitting it out. Women should take rice and milk together for 5 days after taking it. During Pushya Nakshatra, two healthy leaf buds of Vata (Ficus bengalensis Linn.), plucked from the tree's Eastern or Northern branches and grown in a cowshed, should be consumed with cow's curd, along with two seeds of black gram and yellow mustard. Similarly, the paste of Jeevaka (Microstylis musifera.Ridly./ Lipas rostrata.Reld), Rishabhaka (Microstylis wallichi Linn./ Lipas rostrata.Reld), apamarga (Achyranthes aspera Linn.), and Sahachara (Barleria

cristata Linn.) grown collectively or individually. One palasha leaf should be consumed with cow's milk. Using sukashimbi root (Mucuna pruriens Bek.) or the pulp of Pomegranate or Kapittha fruit (Feronia elephantum) or the seeds of Shivalingi (Bryonia Laciniosa), pestle with cow's milk definitely gives birth to a male child [5] and those herbs have been found to be anabolic, steroid positive, and produce testosterone in order to have male offspring. [6]

Maternal blood pressure before pregnancy is a previously unrecognized factor that may be associated with the likelihood of delivering a boy or girl. Changes in maternal vascular function are needed in early pregnancy to accommodate the increased blood flow required by the foeto-placental unit, maternal blood pressure potentially may be relevant to early placentation in a sex-specific manner. While sex-specific attrition may indeed be the mechanistic basis underlying the current findings, it should be noted that our data cannot differentiate between a lesser capacity of the female fetus to tolerate higher systolic pressures or an inability of the male fetus to tolerate lower pressures [7]. During Marsha Nasya, which is the therapy performed to Pumsavana karma, a temporary increase in blood pressure was observed. We believe that incensement of blo od pressure while performing Nasya may influence this sex specific early placentation.

Maternal TSH level in the first trimester is positively associated with the probability of delivering a male newborn. [8] Women pregnant with a Male Fetus have slightly higher TSH levels and a maximum upper limit of the first-trimester TSH reference range than women pregnant with a Female Fetus. Females with high corticosteroids produced more daughters than females with low hormone levels [9] According to the nasya karma mentioned in Charaka samhiha Samhita (withing the pushya Nakshatra, the woman should be made to inhale the vapours of a cake that is being baked on fire, and then the mixture should be cast over the door's threshold. This water should then be applied to the woman's right nostril with a stick of cotton) .Pumsavana-Nasya may stimulate the olfactory nerve, simulates the hypothalamus, and acts on the pituitary glands to regulate hormonal production.

Regarding the comparison between acidic and alkaline vaginal pH and fetal sex, there were significant associations between the fetal male sex and alkaline vaginal pH and a significant association between the fetal female sex and acidic vaginal pH [10],[11]. Pumsavana mentioned, sperm unites the ovum in a clean and healthy genital tract and is home to an embryo with desired characteristics. The cleanliness produced by Pumsavana procedures may have an effect on the pH balance of the female vagina.

A prospective study was carried out to compare the sex ratios of children born to smoking and nonsmoking mothers in terms of parity. The findings suggest that among women who smoked, primi-gravida women have significantly more male offspring than biparous women, whereas women with parity greater than or equal to 3 have significantly more female offspring; biparous women have significantly more male offspring, but the offspring sex ratio decreases with the number of cigarettes when the mothers smoked more than or equal to 10 cigarettes per day. [12] This finding shows that smoking or other health-related behaviors in females can influence gender determination.

The observation that societal stressors like disasters, terrorism, economic collapse [13–16] may reduce the proportion of boys born into a population has led to the "culled cohort" theory [17] which posits that there is a greater spontaneous loss of frail male fetuses in response to such adverse conditions.[18], [19] . In Pumsawana all the ritual are done in the Pushya nakshastra. It is said to neutralize almost all doshas/fundamental energies of the body or flaws caused by a variety of adverse combinations. Pushya, the supreme constellation, has the ability to overcome negative forces and assets its benefic characteristics. Pushya nakshatra, region, time, and the rituals related to Pumsavana have a benefic relationship with maternal psychological health and behaviors.

Female mammalians, according to evolutionary biologists, can reconfigure the sex ratio of their offspring in response to their maternal condition, such that healthy and well-nourished mammals are more likely to give birth to a male progeny. [20]. All research results found seem to align well with Ayurvedic concepts.

IV. CONCLUSION

Every human being contains two elements: masculine energy and feminine energy. Pumsavana sanskara may boost the fetus's masculine energy. The mother's factors determine whether the X chromosome in the ovum is associated with the X chromosome in the sperm or with the Y chromosome during fertilization means certain changes in the mother's body can change the mother's factors that determine the sex of the baby. There could be a considerable influence of Pumsavana karma on fetal sex determination. More research in this field is required to scientifically prove this argument.

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MANAGEMENT OF MANASA DUKKHAJA UNMADA (DEPRESSION) THROUGH A TRADITIONAL TREATMENT MODALITY; A CASE STUDY

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Abstract: Depression is a common and serious medical illness that negatively affect on feelings, thinking pattern and behavior. Globally, it is estimated that 5% of adults suffer from depression. It is a leading cause of disability worldwide and is a major contributor to the global burden of diseases. More women are affected by depression than men which can lead to suicide. Ayurveda considers depression as *Manasa dukkaja unmada, Avasada, Vishada* or *Kaphaja unmada*. Aim and objective of this study was to evaluate role of Traditional treatment in the Management of Depression. A 33-year old unmarried female patient consulted the Neelammahara Manasa Hospital with complains of frequent headache, insomnia, feeling uncomfortable with delusions, inability to feel happiness, feeling sadness always, feeling more lethargy and intermittent aggressive behavior.

The management comprised of *Shirodhara* (Dripping oil on forehead), *Nasya* (inserting medicine through nostrils), *Vireka* (Medicinal Purgation), *Shirsha abhyanga* (Head massage), *Hisa gellum* (Head pack) and internal medicines which are specific to Neelammahara tradition. There was a drastic progress in her condition including good sleep pattern, feeling of happiness reduced feeling of more sadness, no delusional thoughts, calm mind, relaxed body with positive thoughts of her future especially after *shirodhara* and no any adverse effects of the followed management was seen during or after the treatments. Results indicated a 75% improvement in HDRS score pattern showing an excellent reduction of symptoms. It can be concluded that followed treatment protocol is successful in the management of *Manasa dukkaja unmada* which was shown best results related present case study.

***Keywords*:** Depression, Kaphaja unmada, Shiro dhara

I. INTRODUCTION

Depression is a common recurrent disorder and more prevalent in females than males which is characterized by changes of thoughts, feelings and behavior by a negative effect. There are various forms of depression including major depressive disorder, premenstrual dysphoric disorder, persistent depressive disorder, psychotic depression, bipolar affective disorder, seasonal affective disorder and postpartum depressive disorder.

Depression is mentioned in Ayurvedic texts in various scattered references. It is mentioned as a state of emotion (*manasika bhava)* as well as a disease (*manovyadhi*). It can be correlated with *Vishada, Avasada, Manasa dukkhaja Unmada* and *Kaphaja Unmada.*

Among the dreadful psychological disorders, depression takes a major place. In worldwide population also 5-6% people suffer from depression. Interestingly, higher the status of education and profession, higher the life complications, Dissatisfaction and over expectation leads to depression.

Depression is caused by multifactorial combination of genetic, environmental, personality trait factors, gender along with biological component of hypothalamic adrenal pituitary axis, Central nervous system, immune and endocrine system.

Aim and objective of the present study was to evaluate the influence of indigenous treatment modality in the management of Depressive disorder. Current interventions include both pharmacological and psychological aspects to fulfill the indigenous approach in relation with present case study.

II. MATERIALS AND METHODS

A. Case Presentation

A 33 years old female patient admitted to the Neelammahara Manasa Ayurveda Hospital with sign and symptoms of Depression. She complained of frequent headache with insomnia and having no feelings such as happiness or sadness and feeling lethargy.

According to her history, she has had a relationship during her Advanced level education and after breakup she has felt depressive thoughts like suicide. She was suffering from a severe headache and insomnia and intermittent aggressive behavior. After the treatments from Neelammahara she felt better and could spend her life normally.

In 2019, suddenly, she was aggressive and had fights with her family members that she was unable to control her thought flow. She has had delusions such as her family members were going to kill her, her father was a major drug dealer, she will be forcibly abused drugs etc. and therefore she had an anxiety to live in her house. She has had suicidal thoughts several times but, she was

afraid of dying. Due this behavior, she was admitted to the hospital and she was identified as suffering from a lithium deficiency. Then she has undertaken allopathic medicines for a longer period and due to the no progress in her thought pattern she was admitted to the Neelammahara Manasa Ayurveda Hospital.

1) Past Medical History:

Patient was suffering from a lithium deficiency identified since 2019.

2) Past Medication History:

Allopathic medicine in 2015, 2019

Indigenous medicine in 2015, 202

3) General examination:

Weight: 51 kg

Height: 155 cm

BMI: 21.2 kgm^2

Temperature: 98° F

BP: 110/70 mmhg

Physical activity: Daily routine

4) Diagnosis of depression:

The key symptoms (low mood, loss of interest, low energy) of depression were present from last three years.

The given treatment schedule was as per given below Table1.

Table 1. Timeline of case study

	Clinical events and Intervention	**Time duration**
• The patient was admitted to IPD unit in Manasa Ayurveda Hospital. • Based on clinical findings and Laboratory findings, procedure of main therapy including internal and external treatments was advised to continue as below.		
Internal treatments		
1.	*Lunuwila* 12 Decoction (½ cup – Morning & Evening)	
2.	*Wee pori* 5 Decoction (½ cup – Morning & Evening)	
3.	*Pancha Tikta Grhrita Guggulu (*2 pills – Morning & Evening)	
4.	*Manibhadra choorna (1* teaspoonful – at night)	
5.	*Brahmi Pani (*02 teaspoonfulls – Morning and Evening)	
6.	*Kalyanavaleha (*01 teaspoon along with glass of milk – at morning)	08 days
External treatments		
1.	*Shirsha abhyanga* with No. 07 oil	daily

2.	*Shiro dhara* with *Divyanganadi oil*	03 days
3.	*Ushnodaka dhara* with *Savandara & Suduhandun*	03 days
4.	*Hisa gellum* with *Mukunuwenna, Kumbuk kola* and Cow's milk	03 days
5.	*Nasya* with *Anu* oil (6 drops per each nostril)	01 day
6.	*Kiri Vireka* • *Poorva Karma* : *Sarvanga abhyanga* & *Vashpa sweda* for 08 days • *Pradhana Karma* : *Kiri vireka* in 9th day • *Pashchat Karma* : *Samsarjana Karma* for 03 days	12 days
	02 days interval	
7.	*Shiro dhara* with *Neeelammahara maha thela*	03 days
8.	*Nasya* with *Neelammahara Neelakanthi Thaila*	01 day
	02 days interval	
9.	*Shiro dhara* with *Thriphala* oil	03 days
10.	*Nasya* with *Anu* oil (06 drops per each nostril)	01 day

Table 1. Continued

Clinical events and Intervention	**Time duration**
02 days interval	
Internal treatments	08 days
I. *Mee mal* 3 + *Ikini gokatu* 6 Decoction (½ cup – Morning & Evening) II. *Panchatikta Ghrita Guggulu (*2 pills – Morning & Evening) *Brahmi Pani (*02 teaspoonfulls – Morning and Evening)	
Mind relaxing treatments	daily
Pranayama (Breathing exercises) *Nadi shodhana pranayama* Meditation	
Patient was discharged and treated in OPD level	Follow up

Table 2. Hamilton's Depression rating scale: before and after

Symptoms	Before treatment	After treatment
1. Depressed mood	3	0
2. Feeling of guilt	2	1
3. Suicide	3	0
4. Insomnia; early in the night	2	0
5. Insomnia; middle in the night	1	0
6. Insomnia; early hours of the morning	0	0
7. Work and activities	4	1
8. Retardation	1	0
9. Agitation	4	0
10. Anxiety psychic	2	1
11. Anxiety somatic	2	1
12. Somatic symptoms gastro-intestinal	1	0
13. General somatic symptoms	1	0
14. Genital symptoms	0	0
15. Hypochondriasis	0	0
16. Loss of weight	3	3
17. Insight	0	0

B. Patya – Apatya

Light diet, Cow's milk, Fresh fruits and vegetables including apricot, oranges, grapes, potato, tomato, carrot, pumpkin, spinach, nuts and seeds, soup, porridge etc. avoiding more salt and spices and artificial junk foods. Food rich in magnesium and zinc, protein, selenium, vitamin D, E and B6 are much important as dietary modifications.

Relaxation techniques including breathing exercises; *nadi shodhana pranayama* in *Yoga* exercises and practicing meditation for 30 minutes and regular exercises.

III. RESULTS AND DISCUSSION

Results

During and after the treatment a drastic improvement was noticed in the clinical conditions of the patient including good sleep pattern, feeling of happiness, reduction in sad thoughts, no delusional thoughts, calm mind, relaxed body with positive thoughts of her future and her depressive condition reverted back to normal.

Discussion

According to Ayurveda, Depression belongs to *Kaphaja unmada* which is characterized by psychomotor retardation, social withdrawal, reduced self-care, decreased higher mental functions and with confusions, reduced appetite and changed sleep pattern.

Patient has shown three key symptoms; loss of energy, low mood, loss of interest according to diagnostic criteria.

In this case study, combination of *Yukti vyapashraya,* (Pharmacological) *Daiva vyapashraya* (Spiritual techniques) and *Sattvavajaya cikitsa* (Councelling) were used.

Among the external treatment of *unmada roga, Shiro dhara* is one of the best procedures in traditional medicine. It is a procedure which slowly and steadily dripping lukewarm medicated liquid on the forehead of the patient with 4 inches distance on a treatment bed.

The centre of forehead is related to the third eye which is believed that linked with the pineal gland of the body and known as *Agnya cakra* in yoga meditation leads to psycho somatic harmony. The stimulation of this *cakra* stimulates the mind and helps in increasing concentration power with psychic abilities to gain confidence.

Psychotherapy should be given to the patient for instance, cognitive behavioral therapy and interpersonal therapy. Patient should be encouraged to try relaxation techniques and regular exercises which are most important in both body and mind relaxation. Psychotherapy helps to value herself and get involved and make contribution to community activities and suggesting creative or new learning activities or working in social services are beneficial to follow normal lifestyle.

IV. CONCLUSION AND RECOMMENDATIONS

Total score of HDRS revealed marked reduction in score from 29 to 7 up to

the normal range. It has been indicated a 75% improvement in HDRS score according to the reduction of relavent symptoms. After the treatment, complete remission of symptoms such as insomnia, agitation, psychomotor inactivity, anxiety and depressed mood with marked improvement in speech and social communication could be assessed. Based on revealed data, it can be concluded that the present case related *Manasa dukkaja unmada* (Depression) has a drastic positive improvement and the treatment modality is successeful in the management of *Manasa dukkaja unmada.*

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FOCUS AREA

Environment

ORGANIC FARMING MANAGEMENT STRATEGIES

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Abstract: Organic agriculture differs from conventional agriculture in ore integrated with ecosystem functions. Farmers, environmentalists, consumers, and policymakers are becoming ever more concerned with the impact caused by conventional, modern agriculture, and they're wondering if organic agriculture can help solve some of these issues or provide a better future for our food and agriculture systems. Farmers use a variety of biological inputs to improve soil fertility, including animal and green manures, compost, cover crops, and animal byproducts. The nutrients accessible to plants are increased as a result of organic fertilizers & pesticides. In this paper, we examine policy gaps, recommendations, and issues related to a wide range of sustainability domains associated with boosting organic agriculture yields. In this first-of-its-kind integrative analysis, we assessed which options for enhancing yields are likely to have negative side effects. Yield-increasing strategies have both dangers and potential, and the actual effect is dependent on management. that it relies less on off-farm inputs and is m

Keywords: Organic Agriculture, Compost, Biological inputs, Policy gaps, Soil fertility

I. INTRODUCTION

Organic farming has emerged as a potential long-term agro-ecosystem for producing food while ensuring environmental sustainability [1]. Organic farming is one of the alternative agricultural systems for dealing with soil degradation and decreasing soil quality. Healthy soil provides essential nutrients to plants while supporting biotic communities that aid in the soil's defense against environmental degradation. When it comes to soil fertility management, organic vegetable growers face numerous challenges. Organic farming is based on reducing the use of chemical fertilizers, pesticides, and herbicides [2]. These components encourage the decomposition process of plant and animal material, allowing nutrients to be readily available for plants. Soil fertility management with biological inputs ensures that organic agriculture is a biologically dynamic process and very complex

[3]. Interest in organic farming is at an all-time high in the Western world, and many Western countries have developed a reliable organic market. Organic agriculture has the potential to address some of the issues caused by conventional farming while also improving other aspects of our food production systems.

Organic farming has started in 181 countries on 69.8 million hectares of farmland, accounting for nearly 1.4 percent of all farmland on the planet. Organic farmland expanded by 32% and 12% in China and Argentina, respectively, in 2017 compared to 2016. There has been no difference in the rate of growth of organic farmland in the United States, Japan, and Mexico [5]. Government support for organic farming is negligible in most developing countries, and no important organizational policies or programs have been implemented in developing countries. The current study aims to identify extensive factors affecting organic farming in developing countries, identify the best organic farming strategies, and optimize the strategies. Table 1 showed the land extent under organic farming in the world.

Table 1. Region-wise land extent under organic farming in the world as of 2019 [4] [5]

	Country	Land Extent under organic farming(ha)	Total Land extent (million ha)
Africa	Tunisia	306 467	**6.1**
	Tanzania	278 467	
	Uganda	262 282	
Asia	China	3 023 000	**6.1**
	India	1 780 000	
	Kazakhstan	277 145	
	Indonesia	208 042	
	Philippines	200 065	
	Sri Lanka	165 553	
	Thailand	91 266	
	Viet Nam	58 018	
	Pakistan	51 304	
	Azerbaijan	37 630	
Europe	Spain	2 082 173	**14.6**
	Italy	1 908 653	
	France	1 744 420	
	Germany	1 373 157	
	Russian Federation	656 933	
	United Kingdom	497 742	
Latin America	Argentina	3 385 827	**8**
	Brazil	1 136 857	
	Mexico	673 968	
	Peru	315 525	
North America	United States of America	2 031 318	**3.2**
	Canada	1 191 739	
Oceania	Australia	35 645 038	**35.9**
	New Zealand	88 871	
	Fiji	16 604	
	Papua New Guinea	13. 7 5	

Role of organic farming

Organic farming is a production system that protects soil health, ecosystems, and humans [6]. Food security is a multidimensional process that includes components such as availability, utilization, and reliability. Food production persists once all people have constant access to sufficient, safe and nutritious food to satisfy their basic needs for a healthy lifestyle [2]. Organic agriculture is a sustainable and environmentally friendly production system that offers numerous economic, ecological, and social benefits to the world. Despite producing lower yields than conventional agriculture, organic farming systems are more profitable for farmers because consumers are willing to pay more. Organic farming improves soil quality, ensures farm viability, and protects the environment.

Organic fertilizers (liquid & solid) applied to the soil result in much less soil erosion as they absorb more water, and increase soil biodiversity, which also improves long-term agricultural production [7]. Organic fertilizers boost soil health by increasing the soil's ability to hold water, capture nutrients, and promote microbial activity [6]. Organic insecticides don't hurt the environment, but they take time to work because of their gradual action [2]. Organic farming improves food security by increasing pest and disease resistance, and protecting water resources [8]. Organically grown food has significantly higher nutrient levels than conventionally grown food. Organic contains 5–90% more vitamin C and 10–50% more secondary metabolites [9]. Green pesticides, such as garlic, onions, and compost tea, are non-toxic and environmentally friendly. Certain pesticides aid in the identification and removal of diseased and decaying plants in a timely manner, thereby improving crop defense systems. Organic farms' biodiversity increases their resilience to climatic change and weather variability [10].

Use nitrogen more efficiently on organic farms

Soil organic matter, agricultural leftovers, manure, and compost are all sources of organic nitrogen. Organically managed industrial farms use nitrogen more efficiently and are environmentally friendly [7]. In a lengthy field trial in Switzerland [11], the overall nitrogen intake into an organic agronomic crop rotation was 64% of that of an integrated conventional rotation. Nitrous oxides are a significant source of agricultural emissions, and high levels of reactive nitrogen (NH_4^+, NO_3^-) in soils could promote their emission. Organically managed soils emit 492 kg CO_2 equivalents/ha per year less nitrous oxide than non-organically maintained crops [10]. Agriculture's climatic impact in Northern Europe is primarily due to emissions of nitrous oxide (N_2O) from soils and methane (CH_4) from ruminant anaerobic fermentation. N_2O emissions from soils can be reduced by management measures that increase plant nutrient

availability, such as lowering N leaching losses. Organic farming can assist in mitigating the effects of climate change by lowering fossil fuel consumption in cultivation, boosting yields, and improving soil fertility and carbon capture and storage [12]. In organic farming systems, there is a considerable risk of nitrogen loss from organic sources. Other soil disruptions can also affect how quickly green manures decompose. Cover crops and compost could aid in nitrogen management by increasing microbial immobilization and delaying the rate of mineralization [13]. Organic N sources, such as manures, composts, and legume cover crops, can provide adequate crop nutrition to full-season crops while keeping available N levels relatively low for the majority of the growing season. Cover crops must be used to avoid excess N leaching after the growing season [14]. Crop residues from various crops have been shown to significantly contribute to soil N [15].

Some genotypes may produce different grain yields with the same amount of N uptake. Improvements in crop cultivars responsible for effective N utilization may also improve nitrogen use efficiency. As a result, proper understanding, identification, and incorporation of various traits in various crops through various breeding approaches is also beneficial in improving crop nitrogen use efficiency [16].

Animal manure & compost

Fresh animal manure can indeed be composted and spread on the ground for a crop which has not been absorbed into the soil until at least 90 days prior to harvesting a food product. The National Organic Standards Final Rule [3] prohibits farmers from using raw manure. The amount of liquid added, the animal species, and the type of bedding used all influence the nutrient content of manure. Raw manure may have a high concentration of weed seeds, resulting in weed and heavy metal problems. Dried manure is easier to handle and apply to fields uniformly. Heat-drying manure and immature compost may increase ammonia-nitrogen volatilization while decreasing total nitrogen concentration [17].

Composting operations that use windrow composting technology must keep temperatures within the specified range for at least 15 days. Materials must be spun four or five times during this time, with an initial carbon-to-nitrogen (C: N) ratio of at least 25:1 [20]. Many weed seeds and pathogens are killed by the heat created during the composting process. Compost represents only 1%–2% nitrogen by dry weight, so when a crop requires 100–200 pounds of nitrogen each acre, utilizing compost as the major source of nitrogen is hardly feasible [18].

Crop rotation

Crop rotation refers to the process of changing the type of crop planted

on a specific plot of land from season to season. The term "rotation" is used in this manual to refer to both cyclical and noncyclical rotations, in which the same crop sequence is repeated indefinitely on a field to satisfy the farmer's changing business and management goals [20]. Crop rotations are influenced by a number of factors, including soil type and fertilizer input, as well as seasonal rainfall variations. It provides a number of advantages for soils and agricultural systems. All of these reduce the prevalence of weeds, insects, and plant diseases, as well as improve the physical, chemical, and biological qualities of the soil, which are all positive impacts. Improved water holding capacities and aggregate stability are among the physical gains, while an increase in inorganic matter replenishes soil nitrogen (N) and carbon (C) is among the biological improvements. Crops produced in rotation emit fewer greenhouse gases because they consume less nitrogen fertilizer. If cereal crops are planted after a leguminous crop, rhizobacteria can fix atmospheric nitrogen. The market price of a crop is commonly used to determine which crops are utilized in rotations. Despite the multiple benefits of rotating crops, farmers frequently return to continuous crop cultivation, especially when particular crop costs are rising. In the long run, this decision was a mistake. Crop yields have declined in continuous cropping systems, and there have been increases in input demands, leading to lower overall farm profitability [21].

Some rotation crops produce a significant amount of root biomass, which could also contribute to residue. The incorporation of C into stable SOM is also influenced by the quality of crop residue [22]. Incorporating perennial forage crops into crop rotation is one of the most effective ways of increasing SOM levels and improving soil quality.[22] concluded that in soil organic matter management techniques, rotations alternating up to seven years of conventional cropping with at least three years of pasture will keep soil quality within acceptable limits and achieve the objectives of sustainable organic agriculture.

Pest & Weed management

The traditional integrated pest management (IPM) pest control approach in agriculture has relied on a number of techniques. [23] argued for a systems-based approach to pest management. Its goal is to improve the inbuilt operation of agro-ecosystems and inhibit pest issues from arising. Practices frequently contribute to the first two strategies that start to grow healthy plants and stress pests. Inherent plant abilities to suppress pests are better described as reducing plant stress. Healthy soils also have more diverse and active populations of soil organisms.

Crop rotation, a fundamental agricultural practice that began in the early days of agriculture thousands of years ago, aids in the

control of diseases, parasitic nematodes, insects, and weeds [24]. Increased biological activity in soils as a result of extensive use of organic materials could indeed encourage fungal invasion of weed seeds, increasing their rate of survival [25]. Cover crops could really improve soil health and are an important tool for SOM management. Growing a cover crop of corn or barley between annual potato crops could indeed effectively remove Verticillium wilt and reestablish yields to pre-continuous crop production levels [26]. This is a common misunderstanding. In Georgia, fungal disease control for peanuts was achieved through no-till planting in susceptible varieties that were easily killed by glyphosate [27]. Cover crops can have a big impact on soil health and are a great tool for SOM management. Cover crops can also have a significant impact on pest management. The root-knot nematode, Meloidogyne spp., has a very broad host range and is hard to control through crop rotation. Oilseed radish and white mustard were discovered to be effective Heterodera (cyst) nematode trap crops. The population of sugar beet cyst-nematode was limited by 40% after raising a white mustard cover crop in a sugar-beet cropping system on loam sand soils in Poland [28]. Many legume cover crop residues control weeds in the following crop through allelopathy [29]. Undersowing a grain crop with red clover is a practice that can help to form organic matter by producing biomass and controlling erosion [25].

Existing Global policy in organic agriculture

Many initiatives and restrictions in the types of government policies have been implemented around the world to consider the importance of organic farming in achieving sustainable development in the environment. Almost every country in the world is concentrating on the production of organic food. Some of the existing global policies in the world are briefly mentioned in Table 2. Not only that, but also the gaps in the existing global policies and recommendations are briefly described in Table 3.

IPM- Integrated Pest Management

IFOAM International Federation of Organic Agriculture Movements

NGO non-governmental organization

CAAC: Certification and Accreditation Administration of China

CNOPS: China National Organic Product Standard; Sources : [30]

Table 2. Global policy in the Organic farming sector

Countries	Policies	References
Nepal	Farmers' field schools were introduced as successful methods of extension training as	[30]

	part of the IPM program, and they became a pioneering strategy to addressing farmers' problems via public participation. More than 15,000 farmers, including 4,500 women, have been trained around the country. As a result, there has been a 55 percent reduction in pesticide use and a ten percent increase in crop yield.	
Uganda	Uganda has the potential to put over 500,000 ha of land under Organic Agriculture and certify over 500,000 farmers once the sub sector is better regulated and facilitated. Control of Agricultural Chemicals Act in 2006.	[31][31]
China	China established the Organic Food Development Council (OFDC) in 1994 to certify organic products. CAAC (China) was founded in 2002. CNOPS was established in China in 2005. A national mission for sustainable agriculture has been established.	[32][32]
India	In the northeastern part of India, a value chain-based organic agricultural program is being implemented. From 2014 to 2015, India implemented a scheme for integrated horticulture development. The Indian government's "Parampragat krishi vikas yojana" cluster program has brought around 500,000 acres of land under organic cultivation.	[33]
Chile	Chile has two domestic certifying agencies and eight	[34][34]

	international certification bodies. There is now a mechanism in place for voluntary control of organic exports. In 1999, the Chilean government adopted an organic production standard, then in 2006, an organic required rule. Since 2005, a National Commission for Organic Agriculture has been in operation, with business sector participation. In Chile, there is a single organic sector organization that brings together all of the key private sector players. The relationship between the private sector and the government is well-developed.	
Costa rica	The National Organic Agriculture Program was founded in 1999, and the	[34]
	agency collaborated with the industry to build a national strategy for organic production based on participatory discussions. There has been a required organic regulation in place since 2001.	
South Africa	One of the five founding members of IFOAM 1972 was the South African Bio-dynamic Association. In 1993, the first organic farms were certified for export.	
Malaysia	NGOs have been promoting organic farming in Malaysia since the mid-1990s. Agriculture's Third National Conference Organic has been highlighted as a niche market potential, particularly for small-scale farmers,	[34]

	according to policy. By 2010, the government expects the organic business to be worth $300 million and cover 20,000 hectares.	
Thailand	A voluntary government standard for organic farming exists, as well as a government-sponsored accreditation procedure for certification agencies. The central government approved a plan for organic development that includes significant investments in biofertilizer manufacturing	[34]
Denmark	In 1982, supermarket sales began. The government organizes the inspection system, which is now integrated into regular food inspection services. In 1990, a public trademark for organic	[35]
	products was established.	

Table 3. Gaps in organic farming policies and Recommendations

Countries	Policy gaps	Recommendation	References
Pakistan	All relevant stakeholders consider the development of a national organic cotton policy to be a top priority since without one, all stakeholders are uncertain about the future of organic cotton on a national and worldwide level. There isn't a single well-established bio input distribution network to be found. Farmers are	Engagement of important stakeholders/working groups in the implementation of an organic cotton policy Organic cotton farmers will be paid a premium based on the market price of textile industry products on national and international markets, in order to encourage and promote organic cotton production. Establishment of an organic cottonseed multiplying program with an emphasis on short-, medium-, and long-term strategies.	[36]

	unable to obtain loans for organic cotton via one operation due to the legal requirements for obtaining a loan from a commercial bank.	Cottonseed as well as organic cotton sample from the farmer's field are tested for free in a laboratory. For successful control of organic cotton bug, pests, and disease, a "National Organic Certification System" and a bio inputs supply chain must be established. Organic cotton producers can get a specialized credit facility with minimal documentation. Organic cotton regions are being included in the government's priority development agenda. Promotion of organic cotton in hotspots recognized at the national level. Involvement of young people, particularly women, in organic agriculture, organic cotton, and bio inputs businesses in order to create jobs and grow businesses in the region	
Uganda	In Uganda, policy is aimed toward assisting conventional farmers via input programs. Because these programs were not designed with organic farmers in mind, organic producers are effectively supporting their conventional counterparts. Operators have limited	Organic agriculture policy should be consistent with a country's overall agricultural policies in order to give organic agriculture a chance to become mainstream. Encourage the development of organic trading initiatives in the country. The environmental benefits of organic farming should be better recognized so that	[31]

	direction on how to conduct organic produce trading. It's also worth noting that there is really no organic market regulations in Uganda right now. Because organic farmers are still not recognized, it is difficult for them to engage in communication with the government due to a lack of policy.	growers don't just consider the financial benefits.	
Thailand	There is still a lack of collaboration between the private sector and the government.	A permanent bridging body between the government and related organic sector agencies must be established.	[37][37]
	Organic vegetable production adheres to organic production but is not certified organic, as it is primarily intended for domestic use or local markets.	Encourage the growth of organic marketplaces and their integration. Contribute to the development of organic farmer cooperatives' capacity.	
Nepal	The certification procedure is more rigorous, and the expense of organic certification is sometimes a barrier to organic agriculture acceptance, especially for small farms.	Execution of organic standards and certification programs. Lower the amount of organic certification while also empowering producers by allowing them to participate in the development of standards.	[30]
Kenya	There are no special trade procedures or policies in place	Within the region, more effort is needed to promote the East Africa Organic Mark.	[31]

	for organic produce. For small-scale farmers, the certification is still difficult and overly demanding in terms of documents and resources.	Harmonize or create an EAC Certification Body that is accredited. Concentrate on increasing the production of high-demand crops.	
Tanzania	Direct involvement of government agencies in trade may cause issues such as a lack of cohesion and harmonization of policies and services supplied in policy implementation. Lack of openness in the procedures for obtaining the necessary certificates and licenses is also a hindrance to efficient regional trade.	Address the bureaucracy that is currently stifling trade at the border and driving up business costs.	[38]

Challenging issues

There are a number of issues to answer, both theoretically and practically, in order to promote organic farming.

a. Limited access to organic inputs

Imported organic fertilizers are difficult to get at reasonable pricing for farmers. In African regions, organic farmers were unable to obtain improved seeds because they were unavailable in the market. This opens up a market for fake seeds, which have low germination rates and are more susceptible to pests and diseases. As a result of all of this, farmers are only able to use the most labor-intensive features of organic farming, rather than fully exploiting its potential.

b. Infrastructure deficits:

For cross-border organic produce trade to be successful, infrastructure must be in place. The scarcity of such infrastructure creates a trading barrier, especially for perishable goods. Organic producers require cold storage capacity and refrigeration trucks in order to preserve the excellent quality of organic produce that consumers desire.

c. Certification is expensive:

Because there is no policy framework for organic agriculture, organizing value chain approaches for organic products is extremely difficult. Farmers struggle when organic certification is particularly expensive, leading to a shortage of organic farming and marketing policies in the country. Farmers should not name their products as organic until it has received organic certification, and reaching organic markets somewhere else in the region is impossible because they only accept certified produce.

d. Lack of global organic processing.

In both the public and private sectors, the administrative framework and infrastructure for organic agriculture are scarce. To gain access to the international market, quality assurance, credible marketing information, and other services, as well as rules and regulations that comply with those of importing nations, are required

e. Increased consumer costs

Organic items have a higher consumer cost than inorganic products due to the high input cost. People began to turn to inorganic products as a result of this.

f. Problems with maintaining food quality consistency.

Organic products require a high level of food safety, hygiene, and sanitation. It necessitates appropriate agricultural practices, method, and a quality assurance system (services) provided by certified organizations.

g. Lack of awareness among the general public.

The most important being the establishment of own standards and raising awareness among state authorities, organic farmers, merchants, consumers, and other stakeholders.

h. Scarcity of professionals

Furthermore, extension professionals in the country have little and adequate training in organic agriculture, and professional organizations dedicated to organic farming capacity building are still not operational.

Suggestions for organic farm management strategies

Strengthen the organic movements' institutional capacity to lead and coordinate all actors in the sector.

- Interventions at the bottlenecks of whole value chains, with an emphasis on both export and local/regional markets.
- Strategic focus on organic-friendly technology and solutions research.
- Improve the policy climate as quickly as possible to enable further growth in organic production and trade.
- The government should ensure that all relevant players in society are involved. It is taken into

account their role in policy creation and numerous programs.

- Different agencies must be assigned tasks under one primary government agency that is accountable for the proper implementation of policy.
- A permanent bridging body between the government and related organic sector agencies must be established.
- Initiatives for organic food education and awareness among the general public.
- Authorities must establish thorough scientific assessment of policy impacts on the ground level before implementing policy.

Source :[35][30][31]

III. Conclusion

Organic farming practices enhance soil fertility, biodiversity conservation, and reduce nitrogen, phosphorus, and other nutrient losses due to leaching, surface run-off, erosion, and outflow. The future plan for organic agriculture research and innovation must prioritize productivity improvements that address farms as a whole, with special emphasis on ensuring the good ecological function that organic agriculture can bring. In order to compete with the world's food demand, many strategies should be implemented in each organic practice to increase productivity. According to the review, the policy on government and non-government engagement is poor. Farmers have difficulties when organic certification is particularly costly, resulting in a lack of organic agricultural and marketing policies across the country. Before implementing a policy, authorities must conduct a rigorous scientific assessment of the policy's effects on the ground. Strengthen the institutional ability of organic movements to lead and coordinate all participants in the field. It is necessary to establish a permanent bridge organization between the government and related organic sector agencies.

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DESALINATION OF SEAWATER USING VARIOUS SOURCES OF ACTIVATED CARBON

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Abstract: The world demand for drinking water has significantly increased since the world's population has tripled in the twentieth century – which is expected to increase by another 40% - 50% by 2050. Drinking water scarcity is a major problem in the world. As an Island surrounded by the Indian Ocean, it is a great opportunity to purify seawater in Sri Lanka. According to the world water budget, more than 90% of world water resources are oceanic water. Seawater is a good source of water, but its salinity does not allow it for human consumption. As it knows, the major component making sea water saline is NaCl. Therefore, it is necessary to remove NaCl at low cost, and reducing the impacts to the environment.

Many of the currently available water purification techniques for seawater desalination processes are expensive. Thus, the objective of this study is to introduce a low cost highly effective and environmentally friendly method to desalinate seawater, using membrane filtration. Naturally available household and industrial wastes such as coconut shell, sawdust, bamboo and watermelon peel were used as the activated carbon sources for this study. Using batch experiments and column experiments results were obtained. Data were statistically analyzed. According to the characteristics of activated Carbon, the highest Carbon concentration and pore spaces occurred in sawdust. The batch experiments done to different granular and powdered activated carbon, varying the parameters of angular velocity, initial weight, volume, and kept time of the rotating shaker salt removal capacity is high in sawdust. The statistical analysis of (both granular and powdered) activated carbon highest salt adsorption occurred in the sawdust. According to the column studies with *Hydrilla verticillata* in the column led to the effective salt removing capacity rather than the normal or without using the *Hydrilla Verticillata*. Well-developed and organized rotating membrane filter may highly be recommended to further development of this study. This study shows the excellent salt removal capacity or desalination by

using granular activated carbon of sawdust.

Keywords: granular & powdered activated carbon (GAC & PAC); adsorption; membrane filtration; desalination

I. INTRODUCTION

According to the Earth's Water Budget, oceanic water is the large sum of water sources in the earth. Due to the high salinity occurring in the oceanic water it is not directly used for drinking purposes [1]. When considering the annual spatial distribution of rainfall patterns in the globe and Sri Lanka, they are not homogeneous. Thus, water scarcity occurred in Sri Lanka. Many European countries and Gulf Region Countries used Reverse Osmosis to desalinate [2]. That is a very high-cost method. As a developing country which surrounded by sea, low-cost environmental feasible method is more suitable for desalination [3]. Therefore, this study is based to develop such method using membrane filtration process [4].

Objectives

The main goal is to find a feasible solution to desalinate sea water to be used for drinking purpose as a solution of the water scarcity problem in Sri Lanka. The specific objectives of the study were to find a low-cost solution in removing salinity of sea water by filtration and to evaluate the salt removal efficiency of different activated carbon filter media.

II. METHODOLOGY

Purified Coconut shells, Water Melon Peel, Bamboo and Saw Dust separately dried under sun shine and dried sources were heated at 600 ^{0}C, 700 ^{0}C and 800 ^{0}C for 3h separately in a muffle furnace [5]. The resulting mixture of each carbon source was washed separately using 2 mol l^{-1} HCl to remove inorganic impurity. Finally, the black samples obtained was derived into two parts and one sample was dried at 60 ^{0}C and sieved through 2 mm sieve in order to obtained granular activated carbon. Other sample which was dried at 100 ^{0}C sieved through 0.2 mm sieve in order to obtained powdered activated carbon [6].

Turbidity, pH, salinity, cation and anion concentration of the sea water sample were measured using turbidity tube, pH meter, salinity meter and atomic adsorption spectrometer.

First the experiment was done with the coconut shell granular activated carbon.

1 g to 5 g (this mass was taken in order to calculate the mass which need to remove NaCl from 1 l of seawater sample) adsorbent was taken in 20 ml natural sea water sample and kept in rotating shaker (Centrifuge has tubes with 25 ml tubes) for 2 h in 500 rpm. The mixture was allowed to settle for 4 hours and then filtered. Then filtrate

parameters mentioned in the beginning.

Next residual was dried and measured the weight (Dried until constant weight gain at 45 ^{0}C).

Again, experiment was done by changing the volume of sea water sample without changing the weight, time and angular velocity.

Then experiment was performed changing the time of keeping the shaker and without changing the other parameters (weight, volume and angular velocity).

Next changing the angular velocity with keeping all the other parameters (weight, time and volume of the seawater sample) constant.

From all these experiments different parameters were measured and obtained the results.

Secondly, same steps were done to the powdered activated carbon of coconut shell.

Test results ware separately calculated for granular and powdered activated carbon samples of Coconut shell.

Finally, the same procedures were done to the bamboo, saw dust and water melon peel activated carbon. Results were taken.

A glass column (35 mm diameter and 200 mm height) was used to assess the salt removal efficiency at dynamic conditions. Columns were made separately for each granular and powdered activated carbon.

First 20 g of granular activated carbon of coconut shell was sandwiched between sand layers. Secondly 20 g of same activated carbon was sandwiched between both Hydrilla (*Hydrlla verticillata)* and sand layers. Results were collected to measure the different parameters such as cation and anion concentration, pH, salinity [7].

This same procedure was done to other granular and powdered activated carbon samples separately. Varying the weight column studies was done and results were taken.

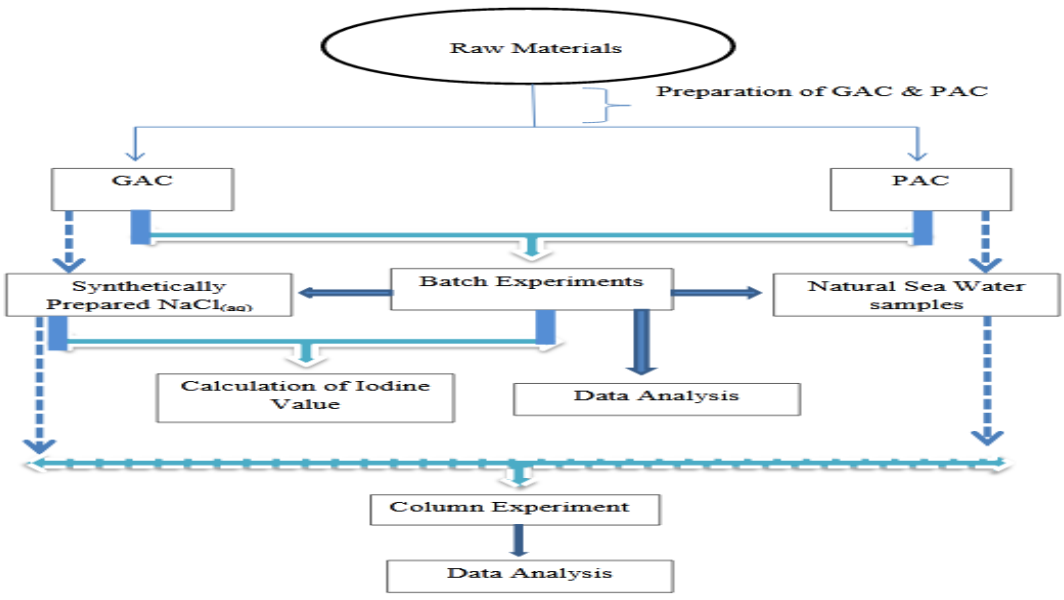

Figure 1. Sketch diagram of Methodology

III. RESULTS & DISCUSSION

Table 1: Basic Characteristics of different Activated Carbon

Source of AC	***Scientific Name***	***Moisture (wt. %)***	***Proximate Analysis (wt. %)***			***Elemental Analysis (wt. %)***					***Ash Analysis (wt. % of ash)***						
			Ash	***Volatiles***	***Fixed Carbon***	***C***	***H***	***N***	***S***	***O***	SiO_2	Na_2O	***CaO***	***MgO***	Al_2O_3	K_2O	Fe_2O_3
Coconut Shell	*Cocos nucifera*	*8*	*2.05*	*28.46*	*69.49*	*80.13*	*2.36*	*1.10*	*0.06*	*16.35*	*37.90*	*0.90*	*4.98*	*1.89*	*24.12*	*0.83*	*15.48*
Saw Dust (Jak Tree)	*Artocarpus heterophyllus*	*10*	*0.64*	*23.66*	*75.70*	*83.75*	*1.23*	*0.30*	*0.05*	*14.67*	*62.87*	*0.035*	*10.35*	*4.18*	*9.85*	*1.71*	*4.45*
Bamboo	*Bambusoideae*	*9.54*	*0.53*	*75.55*	*14.38*	*50.85*	*5.40*	*0.38*	*0.04*	*42.75*	*7.64*	*0.21*	*6.34*	*12.70*	*0.48*	*30.50*	*0.39*
Water Melon Peel	*Citrullus lanatus*	*13*	*13.36*	*72.20*	*14.44*	*45.41*	*6.28*	*0.99*	*0.21*	*47.11*	*69.52*	*1.20*	*2.60*	*1.47*	*10.78*	*19.45*	*0.40*

According to the results of the table 1 it depicts that in elemental analysis, the highest carbon percentage shows in sawdust of activated carbon. Therefore, in proximate analysis, highest fixed carbon percentage (75.70 %) and in elemental analysis highest C percentage (83.75%) observed in sawdust while lowest percentages show in bamboo and watermelon peel. Study further analyzed the least ash oxide percentage in sawdust. Thus, sawdust has the highest adsorption capacity while watermelon peel has lowest capacity.

3.1 BATCH EXPERIMENT

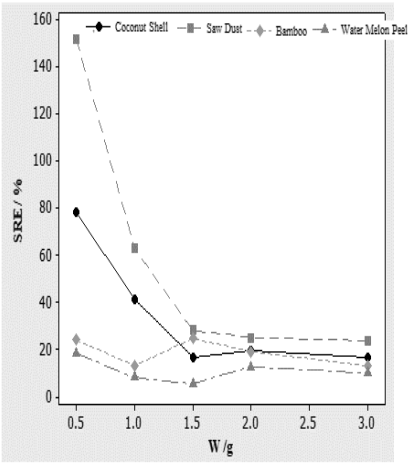

Figure 2(a): M- Adsorbent Weight

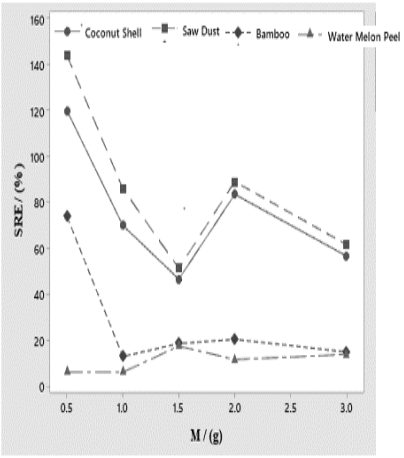

Figure 2(b): W- Adsorbent Weight SRE- Salt removal efficiency

Figure 2(a) & 2(b): SRE of various GAC and PAC when using natural sea water samples.

According to the Figure 2 and descriptive statistics mean values of SRE in natural seawater sample is very high in the sawdust (86.40%) and minimum removal capacity shows in the watermelon peel (10.90%). mean value of SRE of PAC in natural seawater sample is high in the sawdust (58.30%) and minimum removal capacity shows in watermelon peel (10.77%). These mean capacity levels and standard deviations of SRE% in PAC are comparatively less than the mean values and standard deviation values of GAC. Same results were obtained when changing the volume of sea water sample, angular velocity of the rotating shaker and the kept time.

3.2 COLUMN STUDIES

After statistically analyzed the column results using paired and pooled t – test; the following results can be summarized

- SRE was relatively high in Sawdust.
- SRE was relatively high in GAC, SRE can be increased using *Hydrilla verticillata* as a layer in the filter. (82.98 %)

IV. CONCLUSIONS

When compared to the reverse osmosis technique which was the highest efficiency desalination technique in the world [8] (according to the previous studies it was reported that Reverse Osmosis membranes could successfully remove up to 98 % of heavy metals and 99.99 % of NaCl). Membrane filtration process which were used in this study has shown a considerable low cost and high efficiency in desalination process. In column studies or membrane filtration process the ratio of amounts (20 g) used was; Sand: *Hydrilla verticillata*: GAC of Saw dust: *Hydrilla verticillata*: Sand = 1:1:1:1:1. This ratio has the highest desalination effect. SRE was 83%. The response of *Hydrilla verticillata* depends on the concentration of NaCl. More than two months period the material of the column can be used without stagnant and deterioration. Therefore GAC of sawdust have a significant level of salt removing capacity in related to this study. Thus this study shows membrane filtration method using GAC of sawdust was highly effective, environmental feasible way to desalinate the seawater [9].

V. RECOMMENDATIONS

This can be developed up to a filter using a motor inside the column. Rotating the filter will increase the adsorption to the membrane. Therefore, it will be low cost, high efficient, environmentally friendly method to desalinate seawater.

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EFFECT OF MORINGA (*moringa oleifera*) AND JACK (*artocarpus heterophyllus*) LEAVES AS ORGANIC AMENDMENTS FOR TOMATO (s*olanum lycopersicum* L.) CULTIVATION

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Abstract: Chemical fertilizers increase agricultural yields, but they can degrade soil fertility and causing environmental risks. Therefore, a pot experiment was conducted to evaluate and compare the effect of moringa leaves and jack leaves amendments with chemical fertilizers and compost on the vegetative growth, and yield performances of tomatoes (*Solanum lycopersicum* L.) variety Thilina. Six treatments namely T1: 100% recommended chemical fertilizer, T2: 100% moringa leaf, T3: 100% jack leaves, T4: jack leaves 50% + compost 50%, T5: common organic amendment (10 g per pot), T6: control (without any nutrient source) and were replicate three times in a Randomized Complete Block Design (RCBD). T2, T3, T4 and T5 were applied once in two weeks interval up to harvest. Plant height and number of leaves were measured at 2 weeks interval at the vegetative stage and the number of fruits which were harvested from each treatment also measured. The results revealed that the average number of fruits and number of leaves were higher in treatment received jack leaves and compost than chemical treatment. This was on par with other organic combinations. The results suggest that as all organic amendments tested showed the most significant ($p < 0.05$) influence on growth and yield parameters. Therefore, application of the local resources can be an environmentally friendly way to feed the tomato crop to enhance its growth and yield. This experiment concluded that though the application of moringa leaves and jack leaves with compost are helpful in improving yield and growth performances of tomato. Jack leaves 50% and compost 50% as a nutrient source produced better plant growth and yield performances on tomato. The use of those amendments can be both inexpensive and environmentally friendly.

Keywords: *Jack leaves, Moringa leaves, Soil application, Tomato*

I. INTRODUCTION

Chemical fertilizers increase agricultural yields, but they can degrade soil fertility and causing environmental risks. Therefore, to reduces the adverse effect of chemical fertilizer; locally available amendments can be used. In Sri Lanka ample amount of jack leaves are locally available and are wasted. Availability of moringa and jack leaves are high in dry zone. Therefore, there is a need to check its degradability to efficiently utilize as an organic source of nutrients to enrich soils. Researchers found that the growth and yield of crops are increased by using compost. Because it has high bio degradable ability which contain millions of beneficial microorganisms (Manishi *et al*,1996). So, this study was conducted to find the effect of moringa and jack leaves as organic amendments for tomato.

II. MATERIALS AND METHODS

A pot experiment was carried out in Ambanpola divisional secretariat area, Kurunegala district, Sri Lanka (latitude of 7.9181° N, and the longitude of 80.2405° E) during the period from July to September 2021 to study the impact of application of jack leaves, moringa leaves, compost, and chemical fertilizer on growth and yield and attributes of tomato. The tomato seeds were obtained from the Agrarian development center at Ambanpola. This experiment was laid out in a Randomized Complete Block Design (RCBD) with six treatments and three replicates. The treatments were; T1 –100% recommended chemical fertilizer, T2 – 100% moringa leaves amendment, T3 – 100% jack leaves amendment, T4 - 50% Jack leaves+ 50% homemade compost, T5- 100 % homemade compost, T6 -control. 10 g of crushed moringa and jack leaves were added respect to the treatments. The potting media was prepared using topsoil: sand at 1:1. The dimension of a polythene bag was 30 cm x 50 cm. The bags were filled with the potting mixture leaving half- inch at the top to hold the water. Thirty days-old seedlings were transplanted from nursery to each poly bags. All agronomic practices were carried out according to the recommendation by the Department of Agriculture. Leaf amendments and compost application were started at 2 weeks before transplanting for decomposing purpose and it was continued at 2 weeks interval to fruit formation. The data were statistically analyzed using statistical software Minitab 19 and the mean comparison within treatments was performed by Duncan Multiple Range Test (DMRT) at 5 % significant level.

III. RESULTS AND DISCUSSION

Table:1 Effect of different soil amendments on growth of Tomato at harvest values presented are means± standard errors of three replicates. Means followed by the same letter are not significantly difference according to the Duncan multiple range test (DMRT) significance Test at 5% level.
Plant height

Treatments	Plant height (cm)	Number of leaves	Number of fruits
T1	111± 1.52 b	31.6 ± 0.9 cd	8.3 ± 1.5 bc
T2	138.7 ± 3.38 a	48.7 ± 1.8 ab	18 ± 3.5 abc
T3	145.3 ± 1.3 a	50.3 ± 3.9 ab	21.3 ± 5.3 ab
T4	148.5 ± 0.76 a	58.7 ± 1.8 a	31.3 ± 4.1 a
T5	146.3 ± 1.45 a	42 ± 2.3 bc	20 ± 2.3 ab
T6	102.3 ± 3.5 b	20.7 ± 5.7 d	3.7 ± 0.9 c

The results depicted in the Table 1 showed that plant height was significantly increased in all the treatments over control. There is no significant difference between sole chemical T1 and control T6, and also there are no any significant difference among organic manure amendment treatment, T2, T3, T4 and T5. Maximum plant height was observed in application of 50% Jack leaves+ 50% compost, it was followed by compost then followed by sole jack leaves and minimum of level [3]. And this was supported that incorporation of compost normally boosts the number of leaves and leaf area in tomato plants that was for chemical fertilizer and was on par with control plants as an average plant height. organic amendment showed significantly higher height than sole chemical and control treatments, this may be due to the effect of organic amendments jack leaves, moringa leaves, or compost, on soil properties. Compost, unlike commercial fertilizers, releases nutrients slowly and offers both macro and micronutrients. Addition of compost to soil enhances the organic matter and root biomass [4]. The application of organic and inorganic fertilizer had significant effects on plant height. The application of

organic fertilizer combination with cow dung gave significant difference to plant mass (fresh and dry) [1]. When leaf amendment is combined with compost, we can see high-enhanced results which accredited to availability of macro (N) and some micronutrients (boron and zinc) which increased the overall tomato plant height [8].

Number of leaves per plant

The results revealed that the organic amendments significantly increased the leaf number than chemical treatment and control. Significantly highest total number of leaves were observed in pots treated with 50% jack leaves + 50% homemade compost T4, it was on par with T3 Jack alone and moringa alone and significantly minimum was recorded in control and was comparable with sole chemical fertilizer. It may be due to the jack leaves and compost which may enhance soil health for good growth of tomato. That condition proved by the results supported [5], that the compost treatment had the highest values of tomato seedling height, stem diameter, number of leaves per seedling, and total dry matter. Singh and Tiwari (2013) also found similar results regarding the impact of different nutrient sources on the number of photosynthetic leaves in tomato. Organic matter is proven to be better due to its favorable effects on the physical properties of soil [6]. So, jack-leaf amendment and compost cause an increase in the properties of soil. That was supposed for the increase in leaves' number.

Number of fruits per plant

Significantly maximum average number of fruits were observed in 50% jack leaves + 50% homemade compost T4 followed by jack leaves alone T3, and homemade compost T5. The possible reasons for maximum number of average tomato fruits in T4 was attributed to the availability of macro (N) and micronutrients (boron and zinc) by a jack leaves and compost. Sun et al. (2015) sated that application of macro and micro nutrients increases the fruit weight and fruit yield. Control showed minimum average number of fruits. Treatment with moringa leaf alone also had a great influence on fruit weight. This might be due to the presence of cytokinin in moringa leaves which is helpful in increasing number of fruits/plant. These results are corroborated with research findings of Ogbuehi and Agbim, (2018) who reported that the application of 10% moringa leaves increased the number of pods. Leaves are rich source of nutrients such as ascorbate, carotenoids, phenols, potassium, zinc and calcium which enhances the translocation of carbohydrate from the source (leaves) to sink (storage organ). It may enhance the mineral content of the soil due to the organic amendments such as leaves and composts. [10] also reported that mineral nutrition addition to tomatoes increases the yield of tomatoes.

III. CONCLUSIONS AND RECOMMENDATION

This experiment concluded that though the application of *Moringa oleifera* (moringa) leaves and *Artocarpus heterophyllus* (jack leaves) with compost are helpful in improving yield and growth performance of tomato, jack leaves 50% and 50% compost amendment as a nutrient source on tomato produced better plant growth and yield performances. The use of those amendments can be both inexpensive and environmentally friendly. Therefore, it can be recommended that equal combination of jack leaves with compost can be used as nutritive source to get better growth and yield in tomato cultivation.

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FOCUS AREA

Food, Nutrition and Agriculture

CAMAROSA AND FESTIVAL VARIETY EVALUATION OF STRAWBERRY (*Fragaria* species) UNDER CONTROLLED ENVIRONMENTAL CONDITIONS WITH ALTERNATIVE GROWING MEDIA IN DRY ZONE

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Abstract*:* Strawberry (*Fragaria ananas)* belonging to the family Rosaceae, is a commercial fruit crop grown in temperate regions. It is possible to cultivate strawberries in the dry zone under controlled conditions with 23-28°C day and 5-10°C night temperatures. By manipulating the day-night temperatures around 30°C and 23°C, the following research was conducted to identify the best-performing variety among Camarosa(V_1) and festival(V_2) under controlled environmental conditions in the dry zone with substrate treatments; T_1 – Control (50% Compost + 50% Top Soil), T_2 (50% Compost + 50% Coir Dust), T_3 (50% Compost + 50% Sugar Bagasse) and T_4 (50% Compost + 50% Half Burnt Paddy Husk) as growing media. The experiment was arranged in a Completely Randomized Factorial Design. The average number of runners per plant, leaves per plant, plant crown diameter, plant mortality rate, flowering percentage, and duration for flowering were recorded, and size, weight, and Total Soluble Solid were recorded as fruit quality parameters. nitrogen, phosphorus, potassium, and organic matter content (%) were determined through chemical analysis. Data analysis was done using SAS 9.1 version using Duncan Multiple Range Test (DMRT) procedure for mean separation to determine the most suitable treatment combination and variety for strawberry cultivation under controlled conditions in the dry zone. Results revealed the maximum crown diameter (10.93 mm) in T_3 and the maximum number of strawberry leaves (4.6) in T_1. An average of three runners per plant was recorded in November increasing to five in January. Flower bud initiation started in December 2021 and continued until the end of January 2022. The highest amount of nitrogen (31.5 ppm) was recorded in T_3, phosphorus (32.5 ppm), and potassium (86.8 ppm) recorded in T_2. The highest organic matter percentage (34.7%) was in treatment T_3. It can be concluded that the Camarosa variety performed best with 50% Compost + 50% Sugar Bagasse (T_3) treatment in a 1:1 ratio among different rooting mediums for the growth and quality attributes of strawberries.

Keywords: dry zone, growth, fruit quality, rooting media, strawberry

I.INTRODUCTION

Strawberry (Fragaria species) is one of the world's most luscious and soft fruits. It is an aggregate fruit that is highly perishable. It behaves as a perennial herb in temperate climates and as an annual crop in subtropical climates. The presence of seeds in the outer coat of the fruit helps to distinguish it from other fruit crops. Strawberry is a non-climacteric fruit belonging to the family Rosaceae[1].

Low temperatures and modified atmospheres are required to prevent mold growth and fruit senescence, thereby extending strawberry shelf life. It contributes to agricultural system diversification because it is widely cultivated in almost every region of the world, with greater adaptability in warm climate regions[2].

Strawberry is a newly introduced horticultural crop in Sri Lanka. Its distinctive aroma, vibrant red color, juicy texture, and sweetness make it popular. It is widely consumed, either fresh or in prepared foods such as preserved fruit juice, ice cream, jams, chocolates, and milk shakes. Many industrialized food products also contain artificial strawberry aroma.

Strawberry has an increased potential of generating a higher income if it is cultivated in commercial scale. [3]

Plants have evolved ways to respond to environmental signals by changing their growth and development.

A number of environmental factors influence the transition from vegetative to reproductive development in strawberries. For hastening to flower, conditioning treatments for plug plants have been described, such as exposure to certain high and low temperatures and a short-day (SD) photoperiod [4].

It is grown commercially in northern and central Europe, Asia, and America. Strawberry has a wide range of applications in areas near canning plants and kitchen gardens. There have been reports of increased early production when market prices are high, higher quality fruit, better insect, disease, and weed control with less chemical use, lower labor costs, and more efficient water usage under controlled environmental conditions. The current study was carried out to evaluate strawberry cultivars for their growth, fruiting, yield attributes, and yield under net house in controlled environmental conditions[5].

Under a shade (50%) net house, fruit development, flowering, fruiting, yield, and quality parameters performed well

It thrives well in tropical and subtropical climates thanks to the introduction of day neutral cultivars. Varietal performance in a variety of agroclimatic conditions is also important in determining the optimum cultivars for a given agroclimatic condition in order to maximize yield while maintain desirable quality characteristics[6].

Strawberry is grown in a variety of climates, from temperate to subtropical plains to high altitudes in tropical regions even in desert like portions of Israel. Because it is a shallowrooted crop, it is susceptible to both crop damage and plant mortality during dry seasons[7].

II.MATERIALS AND METHODS

Experimental conditions

The study was conducted using plastic pots for cultivation which was placed in a framework under controlled environmental conditions with 50% shade net house, from August (2021) to March (2022) in the Agronomy Department, Faculty of Agriculture, University of Jaffna, Sri Lanka. The particular region was in DL_{3b} Agroclimatic region. The average annual temperature is 30˚C. The average annual rainfall is around 750mm. The rooting media was prepared by using Topsoil, Coir, Sugar bagasse, Half Burnt Paddy Husk, and Compost. The growing media mixtures were placed in plastic pots after sterilization of the media. Physical and chemical analysis for each media was done separately.

Treatments and Experimental Design

The disinfected pots were filled with T_1 (Compost 50% + Top Soil 50%), T_2 (Compost 50% + Coir dust 50%), T_3 (Compost 50% + sugar bagasse (50%), T_4 (Compost 50% + Half Burnt paddy husk (50%). The effect of different growing media under controlled environmental conditions for strawberries is going to evaluate experimentally in the dry zone. Compost and coir dust in a 1:1 ratio are used as control and half-burnt paddy husk; sugar bagasse is used as alternative growing media in the soilless cultivation of strawberries.

Optimizing crop requirements to increase crop productivity can be done by regulating the root zone. [11] Forty transplants of the Camarosa variety and Festiva variety were planted in each pot with different substrates and each pot was irrigated with pure water and treated with Albert solution (30g/L) manually 70ml aqueous solution per day. Treatments were arranged in Completely Randomized

Factorial Design and treatments were repeated ten times among four treatments. Forty (40) pots allocated for this experiment were placed on an elevated iron bench and below the bench was covered with a green cover using buffer grass. A double shade net is used to manipulate shade [12].

Two micro-propagated strawberry cultivars (Camarosa and festival) were obtained from the Fruit crop research center, Rahangala after hardening in April 2021. These plantlets were kept as such for two months in poly bags with a growing media mixture consisting of Topsoil, Coir dust, Sugar bagasse, and Harf burnt paddy husk in a 1:1 Ratio. In August 2021, the plants were again transplanted in well-prepared pots under a polyhouse. At the time of transplanting in pots, the micro-propagated plants were with well-developed root systems and leaves. The planting of experimental material was done on 20th August 2021. Recommended fertilizer and other cultural packages of practices were adopted for better crop growth. Camarosa was the best-performed variety in drought-stress warm conditions[13].

Evaluation of Plant growth

All plants were managed with uniform cultural practices, i.e., Fertigation and irrigation. Observations were recorded per plant. Average plant crown diameter in millimeters with help of a vernier caliper in plants was recorded for each treatment. The average number of runners per plant, the average number of leaves per plant, plant mortality rate, flowering percentage, Duration for flowering, and Duration for fruit-bearing were recorded manually. The fruit quality parameters viz. size, weight, and, TSS, were recorded as per standard methods (AOAC 2010).

Determination of Nutrient Analyses in Rooting Media

The concentration of Nitrogen, Phosphorus, and Potassium nutrient constituents in the different rooting media were determined by laboratory chemical analysis in the faculty of Technology, University of Jaffna, Sri Lanka. According to the methods, analysis of nitrogen in different samples of rooting media was done by proceeding with distillation, extraction, and Titration in the Kjeldahl method. Phosphorus was analyzed by spectrophotometer and Potassium was analyzed by flame photometer.

Statistical Analyses

These data were subjected to statistical analysis (ANOVA) following standard procedures by using SAS 9.1 version and Duncan Multiple Range Test (DMRT) mean separation was done to evaluate the most suitable strawberry variety that can be cultivated under controlled conditions in the Dry zone.

III. RESULTS AND DISCUSSION

Plants have adapted conditions that allow them to change their growth and development in response to environmental conditions. A wide range of environmental factors can affect the transition from vegetative to reproductive development in strawberries. Conditioning treatments for plug plants have been described for prolonging flowering in Short Day strawberry transplants, such as exposure to specific high and low temperatures or low temperatures[4].

Plant Height

Data presented in (Table 1), revealed that growth parameters viz. plant height, and the number of leaves per plant, were significantly different under genotypes. Maximum plant height was recorded under Camarosa (32.5cm) followed by Festival (22.34 cm). The tallest plant (28.67 cm) was obtained in (T_3) sugar bagasse and compost combination followed by (T_4) half-burn paddy husk (18.86 cm) as the shortest (Table 1). In this study overall growth was more in Camarosa. A similar trend was also observed in the production of the number of leaves per plant.

Number of leaves per plant

The number of leaves plant^{-1} was maximum in (T_3) sugar bagasse and compost combination (36.13) and the

minimum were in (T_4) half-burnt paddy husk (25.61) (Table 1). In the case of varieties performance, the maximum number of leaves plant^{-1} was observed in Camarosa (37.25) followed by Festival (35.42) as the lowest. The cultivar

Table 1. Effect of Rooting media and variety on Plant height and Leaves per plant.

Treatments	Plant height cm	Leaves/ plant
Top Soil + Compost (T1)	24.00 [ab]	33.29 [b]
Coir + Compost (T2)	22.27 [b]	30.80 [c]
SB+ Compost (T3)	28.67 [a]	36.13 [a]
HBPH + Compost (T4)	18.86 [c]	25.61 [d]
Varieties		
Camarosa	32.5 [a]	37.25 [a]
Festival	22.34 [b]	35.42 [b]

Camarosa had a maximum number of leaves followed by Festival. The fresh and dry weight of leaves also significantly varied among different genotypes. The cultivars Camarosa had

the maximum fresh weight of leaf followed by Festival. This may be attributed to higher vegetative growth and leaf area in Camarosa which might have increased the accumulation of more dry matter in leaves. Further, the specific leaf area is an important index of leaf structure and it is largely a function of leaf thickness. Moreover, the leaf area and number are phenotypic features of a cultivar which are controlled by the genetic make-up of cultivars and environment. The number of leaves plant^{-1} was maximum in (T_3) sugar bagasse and compost combination (36.13) and the minimum were in (T_4) half-burnt paddy husk (25.61) (Table 1). In the case of varieties performance, the maximum number of leaves plant^{-1} was observed in Camarosa (37.25) followed by Festival (35.42) as the lowest. The cultivar Camarosa had a maximum number of leaves followed by Festival. The fresh and dry weight of leaves also significantly varied among different genotypes. The cultivars Camarosa had the maximum fresh weight of leaf followed by Festival. This may be attributed to higher vegetative growth and leaf area in Camarosa which might have increased the accumulation of more dry matter in leaves. Further, the specific leaf area is an important index of leaf structure and it is largely a function of leaf thickness. Moreover, the leaf area and number are phenotypic features of a cultivar which are controlled by the genetic make-up of cultivars and environment.

Table 2. Effect of Rooting media and variety on Plant mortality percentage and Crown diameter

Varieties	Plant mortality %	Crown Diameter mm
Camarosa	10.1 [a]	13.11 [a]
Festival	21.2 [b]	09.87 [b]

Plant mortality %

The highest temperatures recorded in high tunnels were above 30˚C for about a week in mid of August, which caused 21.2% of death of 'Festival' plants, and 10.1% death of 'Camarosa' plants. Dead plants were replanted in the first week of September 2021. It should be noted that the temperature inside the net house can reach up to 28˚C when temperatures outside of the high net house were above 30˚C. The lowest temperature recorded in the rainy season was about 23˚C, at the time when the temperature outside of the high net house was about 25˚C.

No crown damage was observed, while flower damage was noticed on varieties that had open blooms in the rainy season.

Crown Diameter

An increase in crown diameter means more vegetative growth i.e., an increase in the number of lateral branches, flowering trusses, runners, and leaves [14] Maximum crown diameter (10.93 mm) was obtained in plants grown in Sugar Bagasse(T_3). The minimum crown diameter (7.20 mm) was produced by coir dust in (T_2).

Runner production

Pruning of runners is necessary to ensure plants direct energies to crown development. Vegetative growth in the form of runner production averages

three runners per plant in November, increasing to about five in January. In contrast, the 'Strawberry Camarosa' strawberry plants that were subjected to the same propagation scheme but were in four different rooting media under shade nets in August did not flower until January. The runners resulted in an average of three runners per plant in November increasing to five in January and then no more in the following months.

The number of runners per plant and crown growth was also significantly influenced due to cultivars under polyhouse conditions (Table 3).

Table 3. Effect of rooting media on runner production in Strawberry (Festival variety)

Festival			
Treatment	**Nov**	**Dec**	**Jan**
Top Soil + Compost (T1)	1.5 [b]	2.7 [b]	4.9 [b]
Coir + Compost (T2)	0.2 [a]	1.5 [a]	3.4 [a]
SB+ Compost (T3)	1.8 [b]	3.2 [b]	5.0 [b]
HBPH + Compost (T4)	0.4 [a]	1.7 [a]	3.6 [a]

Table 4. Effect of rooting media on runner production in Strawberry (Camarosa variet

Camarosa			
Treatment	**Nov**	**Dec**	**Jan**
Top Soil + Compost (T1)	3.2 [b]	4.0 [a]	5.0 [b]
Coir + Compost (T2)	2.0 [a]	4.3 [a]	5.1 [a]
SB+ Compost (T3)	7.2 [b]	8.1 [b]	**9.4 [b]**
HBPH + Compost (T4)	5.1 [b]	6.3 [a]	7.1 [a]

The highest number of runners was recorded in Camarosa (9.41/plant)

followed by Festival. A similar trend was observed in crown development during crop growth. In a study by [15], the runner production in cultivar Festival was comparable to several other commercial cultivars grown under greenhouse conditions in central Florida. However, in the present study, it was observed that the strawberry cultivar Camarosa had *vigorous* and larger plant size as compared to other cultivars which might be due to a greater number of runners per plant. The type of media used did not significantly affect the number of runners in the strawberry cultivar Festival when grown under greenhouse conditions [16]. Root and crown dry matter production were more in cultivars of California origin i.e., Chandler and Camarosa, whereas, Sweet Charlie, a cultivar from Florida, showed lower dry matter accumulation and relative growth rate[5].

Flowering percentage

Runners were counted and removed at the end of November, December 2021, and January 2022. Flower bud initiation was started in December 2021 and it was continued until the end of January 2022.

Table 5. Effect of rooting media on flower production in Strawberry (Camarosa variety)

Festival			
Treatment	**Nov**	**Dec**	**Jan**
Top Soil + Compost (T1)	00 [a]	10 [b]	27 [c]
Coir + Compost (T2)	00 [a]	00 [a]	00 [a]
SB+ Compost (T3)	31 [b]	47 [c]	53 [d]
HBPH + Compost (T4)	00 [a]	00 [a]	10 [b]

Table 6. Effect of rooting media on flower production in Strawberry (Festival variety)

Camarosa			
Treatment	**Nov**	**Dec**	**Jan**
Top Soil + Compost (T1)	21 [a]	30 [b]	48 [c]
Coir + Compost (T2)	00 [a]	00 [a]	00 [a]
SB+ Compost (T3)	40[a]	**62 [b]**	**70 [b]**
HBPH + Compost (T4)	00 [a]	00 [a]	10 [b]

strawberry Festival was the earliest to flower, followed by Camarosa (data not given). Camarosa and Festival (3.80 and 0.81 berries/plant, respectively) gave a significantly higher number of berries per plant over the other two varieties. The maximum production in Camarosa might be attributed to the maximum berry size (11.53 g) as compared to Festival cultivars (5.89g).

Fruit quality

The Strawberry scent and titratable acidity are both characterized by the TSS concentration, which is also directly regulated by genetic and environmental variables. A fruit's flavor is mostly influenced by the interaction between its total acid and TSS contents and these two elements [17].

Brix value may vary between cultivars and cultivation sites; as an example in a conventional cultivation system in Mexico, the average ranged from 8.4 to 9.0 Brix [18], and for Albion cultivated in a protected environment in China [19] observed values and 8˚ Brix.

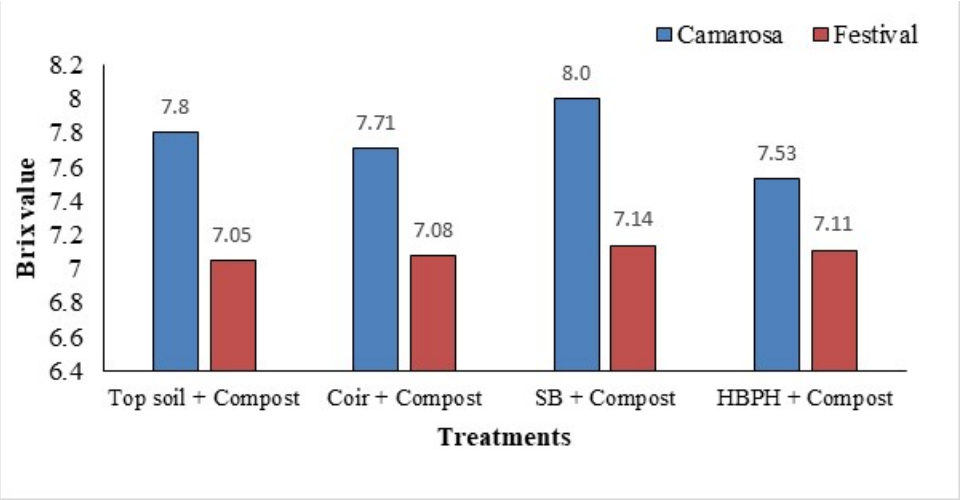

Figure 1. Effect of Strawberry varieties on brix value

Nutrient Analysis of Different rooting media

Table 7. Nutrient composition of different rooting media

Treatment	Nutrient level			
	N content (ppm)	P content (ppm)	K content (ppm)	Organic matter content %
Top Soil + Compost (T1)	11.76	17.8	37.35	5.5
Coir + Compost (T2)	22.68	**32.5**	**86.8**	8.29
SB+ Compost (T3)	**31.5**	2.9	77.15	**34.7**
HBPH + Compost (T4)	26.04	14.5	52.9	27.29

The results indicated that there was a significant difference found in nutrient levels due to the effect of rooting media (Table 3.4). Rooting media of Sugar Bagasse + Compost (T3) was the meantime P content (32.5 ppm) was also significantly higher at Coir + statistically significant at ($p<0.05$) and the highest amount of Nitrogen (31.5 ppm) was recorded. The highest Potassium content (86.8 ppm) was observed in the Coir + Compost (T2), in Compost (T2) than in other rooting media. It was significantly influenced

by the Nutrient retention capacity of coconut coir dust which determines physical properties, such as aeration and water supply and availability, which are greatly dependent on particle size[20]. Organic matter percentage (34.7%) was highest among applications of SB+ Compost (T3)[21]. A significant amount of Organic matter (27.29%) was present with the HBPH + Compost (T4)[17]**.** By considering the above results it can be concluded that strawberry plants perform better on the Sugar bagasse with compost in a 1:1 ratio based on rooting medium for growth and quality features of Strawberry in productivity.

IV. CONCLUSIONS AND RECOMMENDATIONS

The results of the current study indicated that the cultivar Camarosa produced the best growth, production values, and Strawberry fruit quality when grown in organic compost combined with sugar bagasse. It is important to consider the substrate mixture's physical properties . From the findings of this study, it can be identified that substrates with ideal physical properties can have a significant impact on strawberry plant production and in some cases, offer local farmers affordable alternatives to more traditional substrates, increasing the net profitability of the cultivation system. The growth of the leaves, cro wns, roots, flowers, and fruits of "Cam arosa" and "Festival" strawberry plants throughout
the time followed a successful showin g in a 7month observation period in Kilinochchi, Dry zone.
According to the results obtained from the duration of flowering and fruiting, we can conclude that there's a chance for strawberry seasonal bearing in the dry zone.

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Influence of different processing and drying approaches in the preparation of palmyrah tuber flour utilized as raw material in food industries

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Abstract: This study investigated the influence of different heat treatments such as pre-cooked, un-cooked on the drying behavior of the resultant tuber flour source obtained from Palmyrah palm (*Borassus flabellifer*), which is potentially valued as a food ingredient for the industry. The treatment type (Steamed, boiled with 1% NaCl, boiled without NaCl) and drying air temperature (34°C, 60°C, 80°C) also greatly ($p<0.05$) influenced the yield of the tuber flour. Functional, physio-chemical properties of the flours were determined. The uncooked drying at 60°C flour (79.82%), and boiled with 1% NaCl drying at 60°C flour (78.45%) had a high amount of yield% and low amount of moisture content (3.77%, and 3.43%) respectively. The flour had a high amount of crude fiber (2.62 - 3.34%), and carbohydrate (85.22 - 87.83%). The high fiber content can be explored in the production of pre-biotic foods, relatively high-water absorption capacity (181 – 303%) swelling power (2.87 – 10.05%), and oil absorption capacity (105 – 151%) also implies tuber flour could be widely applied in pastry industry as a thickener, binder, and stabilizer. The low foaming capacity (3.72 - 10.21%) also makes tuber flour a possible component in the biscuit industry. The different heat treatment methods had no significant effect on the pH (6.14-6.82) of the tuber flour. These results recommend that the heat-treated tuber flour can be applied in the production of bread, cookies, cakes, gravies, pastry, pasta, and soup mix due to its high swelling power and water absorption capacity. However, out of the total five selected districts, Mannar district has the highest yield ratio and low moisture content. Comparatively the use of boiled with NaCl and drying at 60°C heat treatment and raw tuber drying at 60°C heat treatments existing the best option considering the cost, drying temperature, and time on the yield of tuber flour and quality factors.

Keywords: **palmyrah, un-cooked, pre-cooked, tuber flour, functional, physio-chemical**

I. INTRODUCTION

Flour is used as raw material for various products of starch and sweeteners in food industries [1]. Starch is used as a taste enhancer, binder, filler, thickening agent, and stabilizer. The fresh Palmyrah *(Borassus flabellifer*) tuber is seasonally available particularly from December to

March per year [2]. The raw and [2] cooked Palmyrah tubers have been consumed and used as a raw material for food preparation [3]. During the fruiting season of September to October.

Palmyrah palm can bear 200-300 fruits which often contain 2-3 seeds. After the pulping process, seeds are collected and beds are prepared with 3-4 layers of seeds with the maintenance of moisture level. During germination, the germination axis of the seed goes to the ground and the distal part that remains inside the seed grows into the scavenger during and after germination. Palmyrah haustorium is a delicious white spongy edible part, and the seed produces a sprout that gives rise to the palmyrah tuber product. Typically, Palmyrah tubers are harvested at their fully mature stage at end of four months period. Tubers contain a lot of nutrients, phenols, and metal ions that exhibit antioxidant properties. The seed kernel contains a galactomannan.

Upon generation, the seed produces a sprout (fleshy food-preserving flakes) which gives rise to the product *panankilangu* (kottaikilangu), boiling and drying or drying alone. This shoot grows up to 12-15 cm in height before being harvested. If the germinated seed is dried in the sun, it is called *Odiyal* (un-boiled tuber). The sprout can be boiled and dried, in which case it is called *pulukodiyal* (boiled tuber). The tuber is eaten directly by many people by cooking it over an open fire after peeling off the outer layer. The boiled and unboiled tuber can be ground and sieved to obtain palmyrah flour (*odiyal* flour or *pulukodiyal* flour).

The Palmyrah tuber is an inexpensive source of starch, carbohydrates, lipids, some minerals, and some health-promoting bioactive compounds [4]; [5] which is useful in controlling various diseases, especially diabetes. Regular consumption of palmyrah tuber flour increases body strength, and reduces hunger, and mixing palmyrah tuber flour with other foods is said to positively reduce malnutrition. Palmyrah tuber flour is not widely consumed by people, but the excellent nutritional and therapeutic value of this palmyrah tuber flour offers great potential for processing into various quality products. As nearly 5,000 tons per year, of tubers, are used for the production of value-added products [6].

The suitability of any post-harvest food treatment depends on its effect on the nutritious and functional properties of the food [7]. The application of different processing methods to 'palmyrah' tubers may influence the physical and functional properties of flour. And at present, they are preserved by either sun-dried (raw dried tubers) or boiled. Sun drying is a common food preservation method that leads to nutritional loss and is time-consuming in processing, hence the quality and storage stability of tuber is poor. Therefore, boiling and drying heat treatments have been adopted to preserve nutritional composition and extend the shelf life by maintaining the moisture content of these seasonal products.

The appropriate and successful incorporation of flour into food products depends on the physio-chemical, functional, and nutritional composition [8]. Therefore, the proximate composition, functional properties such as water binding capacity, foaming properties, and swelling power of flours are usually analyzed to predict their suitable incorporations and explorable applications in food systems. Previously, research has been conducted on the effect of precooking and air temperature on the drying kinetics and quality of tuber flour using hot air drying [9]. And different studies have been conducted on the analysis of the physical and chemical properties of tuber and palm flour [10]. And so far, no research has been conducted to compare different types of processing such as boiling, steaming, and hot air oven drying with the quality of palmyrah tuber flour. Considering these shortcomings, the present study was planned to investigate the effect of different heat treatments on the drying behavior and the physio-chemical and functional properties of the resulting flour for effective use as a food ingredient in processed products.

II. MATERIALS & METHODS

2.1. Sample collection

Freshly harvested palmyrah tubers were collected by retaining the convenient sampling method from the different seedbed growers in Jaffna, Vavuniya, Mannar, Kilinochchi, and Mullaitivu districts in the Northern Province.

2.2. Sample preparations of palmyrah tuber

Freshly purchased *Borassus flabellifer* tuber was subjectively evaluated. The length of the root was measured using a fixed vertical measuring rule by which the root was made to stand vertically and the length measured. Tubers were cleaned and the outer skin was removed. The fresh palmyrah tubers were thoroughly washed with tap water for 5 minutes and then with potable water 3 times to remove soil and sand. And the excess water was drained off. After peeling, the average weight and diameter of whole tubers were taken. The diameters of the roundish bottom of the tuber were respectively measured using a vernier caliper. The average weight of the whole tuber was determined by weighing it before peeling and after manual peeling. Then the tubers were divided into four portions and each portion contained an equal number of tubers.

The selected tubers were subjected to four levels of pretreatment including three types of precooking such as steaming at 121°C for 20 minutes, boiling with 1g of NaCl at 100°C for 30 minutes, boiling without NaCl at 100°C for 30 minutes separately, and uncooked (raw). The surface fiber of the pre-cooked tubers was removed by using the knife and the tubers were cut into thin sections crosswise and dried under three different drying conditions namely, sun drying for three days (36 hours) from 10 am to 4 pm during March and the temperature nearly (30°C - 34°C) as it is practiced in household level, convection drying oven (GEMMYCO YCO – IN 010, Germany) at 60°C for 8-10 hours,

convection drying oven (GEMMYCO YCO – IN 010, Germany) at 80°C for 5-7 hours. The surface fiber of the uncooked tubers was removed by using the knife and those tubers were cut into thin sections crosswise and allowed to dry under three different drying conditions as mentioned
above.

2.3. Palmyrah tuber flour production and storage

The dried tubers were then ground using a hammer mill [5]; [6] and sieved through a 250µm sieve. Tuber flour was packed in low-density polyethylene (LDPE) bags and kept at the atmospheric condition for further studies. All the other conditions were kept similar.

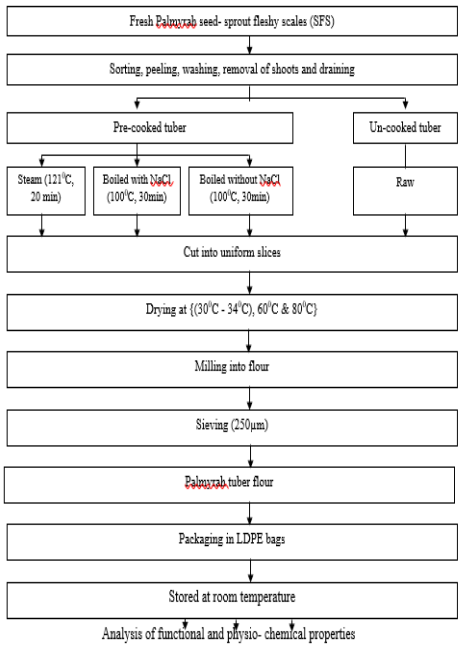

Figure1. Flow chart of the experimental design

The treatments were indicated as T1- Raw sundried for three days, T2- Raw oven dried at 60°C for 10hrs, T3- Raw oven dried at 80°C for 5hrs, T4- Steam sun-dried for three days, T5- Steam oven dried at 60°C for 10hrs, T6- Steam oven dried at 80°C for 5hrs, T7- Boiled with 1% NaCl sundried for three days, T8- Boiled with 1% NaCl oven dried at 60°C for 10hrs, T9- Boiled with 1% NaCl oven dried at 80°C for 5hrs, T10- Boiled without NaCl sundried for three days, T11- Boiled without NaCl oven dried at 60°C for 10hrs, T12- Boiled without NaCl oven dried at 80°C for 5hrs.

2.4. Functional properties of palmyrah tuber flour

2.4.1. pH: The pH of the flours was measured in a 10% (w/v) dispersion of the samples in distilled water. The suspension was homogenized and the samples were allowed to stand for 30 minutes. The pH reading was measured using a digital pH meter calibrated with pH 4.0 and 7.0 buffer solutions, by direct immersion of the electrode into the beaker containing suspension, according to the methodology proposed by the AOAC (2000) method.

2.4.2. Yield: It is calculated based on the flour obtained from the specified quantity of tuber under different heat treatments. Multiply the wet weight by the dry percentage to calculate the dry matter yield [11].

$$Yield\ \% = \frac{Edible\ product\ weight}{Raw\ weight} \times 100$$

2.4.3. Bulk density: This was determined according to the method of [12](Musa *et al*.,2011) with slight modifications. A 20g of flour was taken in a 100 ml graduated glass cylinder using a short-stemmed glass funnel. The volume occupied by the flour was read and the bulk density was calculated in grams per milliliter.

2.4.2. Tapped density: It was determined according to the method of (Musa *et al*.,2011) [12]. A graduated cylinder containing 20g of flour was dropped on a bench 50 times from a height of about 20mm and the respective volumes were recorded. The same was done for all samples and the tapped densities were then calculated in g/ml.

2.4.3. Water absorption capacity: It was determined as described by Sosulski *et al.,* (1976) [13]. One gram (1 g) of flour sample was taken and mixed with 10 ml of distilled water to determine the water absorption capacity. The mixtures were allowed to stand at 30+2 °C for 1 hour. It was then centrifuged at 3,500rpm for 30 minutes (Gemmy-Harmonic series PLC).Water absorption capacity was expressed as the percentage of water absorbed by the flour.

2.4.4. Oil absorption capacity: Oil absorption capacities were determined as described by Sosulski *et al.,* (1976) [13]. One gram (1 g) of flour sample was taken and mixed with 10 ml of oil to determine the oil absorption capacity. The mixtures were allowed to stand at 30+2 °C for 1 hour. It was then centrifuged at 3,500rpm for 30 minutes (Gemmy-Harmonic series PLC). Oil absorption capacity was expressed as the percentage of water absorbed by the flour.

2.4.5. Foaming capacity (FC) and foam stability (FS):
Foam capacity (FC) and foam stability (FS) were determined by the mentioned method in Narayana and Narasinga Rao, 1982 [14]. Two grams of flour were added to 50 ml distilled water at 30 ± 2 °C in a 100 ml measuring cylinder. The suspension was mixed and properly shaken for 5 minutes to foam and the volume of the foam after the 30s were recorded. The FC was expressed as a percentage increase in volume. The foam volume was recorded 1 hour after whipping to determine the FS as a percentage of the initial foam volume.

2.4.6. Water solubility (WS) and swelling power (SP): Solubility and swelling power determinations were carried out based on a modified method of Sanful *et al.,* (2013) [15]. A 0.20 g of flour was dissolved with distilled water to a total volume of 8 ml using a weighed 10 mL graduated centrifuge tube. The suspension was stirred just sufficiently and uniformly avoiding excessive speed since it might cause fragmentation of the starch granules. The slurry in the tube was heated at 85°C in a thermostatically regulated temperature water bath for 30 min with constant gentle stirring. The tube was then removed, wiped and dried on the outside, and cooled to room temperature. It was then centrifuged (Gemmy -Harmonic series PLC) at 2200

rpm for 15 min. The supernatant was decanted into a pre-weighed moisture can. The solubility was determined by evaporating the supernatant in a thermostatically controlled drying oven at 105°C. The sediment paste was weighed and swelling power was calculated as the weight of sediment paste per gram of flour used.

2.5. Physiochemical analysis of palmyrah tuber flour

The compositions such as moisture, total fat, protein, ash, and crude fiber of the different heat-treated boiled tuber flour and un-boiled tuber flour were determined by using AOAC (2000) standard analytical methods. Carbohydrate content was determined by estimation using the arithmetic difference method (D.A. Pearson 1976) [%CHO = 100 – (% Fat. + % Ash + % Fiber + % Protein+ %Moisture)].

2.6. Statistical analysis

The treatment results on various parameters were statistically analyzed using analysis of variance (ANOVA). All the tests are carried out for three replicates. The mean range ± value was calculated using standard deviation. Statistical

significance was determined at a 95% confidence level using the statistical software MINITAB 17.

III. RESULTS & DISCUSSION

Table 1. Weight distribution of palmyrah tuber

District	Average weight of whole tuber(g)	Average weight of peeled tuber(g)	Average height of the tuber(cm)	Average width of tuber(cm)
Mannar	73.63±17[a]	67.74±20[a]	25.6±10[a]	2.92±1[a]
Mullaitivu	68.83±12[b]	60.41±15[b]	22.34±10[b]	2.64±1[b]
Jaffna	69.91±12[b]	61.19±15[b]	23.39±8[b]	2.55±1[b]
Vavuniya	61.79±13[c]	56.05±15[c]	22.89±8[c]	2.41±1[c]
Kilinochchi	29.17±8[d]	24.59±12[d]	19.81±6[d]	1.94±1[d]

Each value in the table is represented as mean ± SD (n=3). Values are significantly different (p<0.05).

This study observed improving the flour quality of palmyrah tuber. Palmyrah tubers have undergone different processing techniques. The palmyrah tuber is an under-exploited resource, that is abundant during the particular season and cheaper to buy in local markets.

3.1 Physical properties of palmyrah tuber flour

The highest average weight of the tuber before and after it peeled was shown in Mannar district while it was observed lower in Kilinochchi district (Table 1). However, Mullaitivu and Jaffna districts showed nearly in between 56g-61g of average peeled tuber weight. On the

other hand, the Average length and width of the tuber were significantly lowest followed by the Kilinochchi district. Significantly highest tuber height and width were obtained from Mannar.

3.2 Weight loss percentage

Weight loss was shown from 19% to 65% for different processing methods. According to the results obtained, T3 (Raw oven dried at 80°C for 5hrs), and T6 (Steam oven dried at 80°C for 5hrs) showed higher weight loss in all districts while it was observed lower in T10 (Boiled without NaCl sundried for three days). Significantly lower weight loss% was observed in the Mannar district (19-39%). The highest weight loss% was recorded in the Kilinochchi district (32-65%).

Higher yield% observed in Mannar district un-cooked treatment T2 (Raw oven dried at 60°C for 10hrs), and pre-cooked treatment T8 (Boiled with 1% NaCl oven-dried at 60°C for 10hrs). Significantly lowest yield% was obtained in T12 (Boiled without NaCl oven dried at 80°C for 5hrs). Jaffna, Mullaitivu, and Vavuniya districts' yields were the same under different heat treatments.

Compared with the other districts, Mannar district has shown the highest yield% approximately 70%. The lowest yield% was observed in the Kilinochchi district around 15%. Furthermore, Mullaitivu, Vavuniya, and Jaffna districts stayed the same yield between 30%. Out of the total five districts, Mannar tuber flour was selected for further analysis as it has the highest yield ratio and low moisture content.

Variations in tuber weight, width, and length distribution were assessed in different districts. Significantly, among these, the district of Mannar was found to have the greatest weight, width, and length. In Kilinochchi district, a high percentage of weight loss was observed in pre-cooked and un-cooked samples as compared with samples from other districts. palmyrah can grow in sandy, alluvial, red latosols and calcareous soils (Monograph). According to the soil geographic pattern, the districts the soil type in Jaffna, Killinochchi, Mannar, Mullaitivu, and Vavuniya were calcic red-yellow latosols, Alluvial soils with red-yellow latosols, regosols sandy soils, reddish-brown earth low humic clay soils and reddish-brown soil respectively.

Table 2. physio-chemical and functional properties of commercial tuber flour

Physio-chemical & functional properties	Commercially available un-boiled palmyrah tuber flour	Commercial boiled palmyrah tuber flour
Moisture%	8.32±0.07	10.10±0.33
Ash%	2.24±0.03	2.07±0.03
Crude fiber%	4.62±0.03	3.28±0.06
Fat%	0.49±0.01	0.37±0.02
Protein%	3.19±0.15	1.47±0.11
Bulk density (g/cm^3)	0.40±0.01	0.70±0.01
tapped density (g/cm^3)	0.50±0.00	0.73±0.06

foaming capacity %	75.67±0.58	16.00±2.00
Foam stability%	2.00±0.00	2.00±0.00
Water Absorption Capacity%	160.00±0.00	260.00±0.00
Oil Absorption Capacity%	140.00±0.00	60.00±0.00
Solubility%	16.80±0.95	13.19±0.0.65
Swelling power%	16.50±0.15	11.80±0.10

Yield and moisture are important factors for the flour manufacturing industry as it increases productivity and hence increases the production and surplus of the industry. On the other hand, the moisture content is an indicator of the storage capacity of the flour. The results of this study showed that uncooked oven dried 60°C for 10hrs (T2), boiled with 1% NaCl oven dried at 60°C for 10hrs (T8) flour samples had higher values of the yield% than the other heat treatments. It may happen due to the medium heat evaporating moisture without case hardening of the tuber. Increasing temperature in a hot air oven results in a decline in drying rate. This is done because of the higher mass fluidity of the water molecules from the inner part to the surface. This is followed by hurried evaporation from the surface to the bulk air.

Significantly lower yield% was obtained in boiled without 1% NaCl oven dried at 80°C for 5hrs. Treatment type had a significant impact ($p<0.05$) on the yield, with the uncooked samples recording higher values (ranging from 64.44% to 79.82%) than the pre-cooked ones (ranging from 62.44% to 78.45%). Likewise, even without a specific trend, districts had a significant impact on yield%. The highest level of yield% was observed in the Mannar district (70%) and the lowest yield% was observed in the Kilinochchi district (15%).

Tubers are susceptible to deterioration at harvest and during storage due to their high moisture content (~75%wet basis). Drying is one of the most common ways to extend the shelf life of food and agricultural products by reducing moisture level to a lower water activity level which inhibits microbial and quality deterioration and reduces the storage and transportation for utilization without any seasonal limitations. According to the results obtained, the oven drying method is more appropriate because the oven drying method reduces the moisture within a shorter period and therefore it is time effective. If the moisture content exceeds more than 14.5%, this favors microbial growth. Here, palmyrah tuber treated with boiled with 1% NaCl oven drying at 60°C for 8-10 hour method is more suitable to reduce moisture content which helps extend the shelf life of the flour.

However, Sahni *et al.,* (2014) [16] reported that the moisture content for roots was 62.38%. It is noticeable that

the drying of tubers is quite effective in causing an ultimate reduction of the moisture content to very low levels that ensure the long shelf life of the product. However, the tuber which was steamed and sun-dried had shown moisture content of 10.6%. The highest reported moisture content was recorded in T2 (Raw oven dried at 60°C for 10hrs) Kilinochchi district.

Remarkably, the lowest moisture content was obtained from T9 (Boiled with 1% NaCl oven-dried at 80°C for 5hrs) in the Vavuniya district. It may happen due to the initial Yield of tuber in Vavuniya being less. Based on comparison with the available results shown, the moisture content% of heat treatment goes as follows, steamed > boiling without NaCl > boiling with 1% NaCl > un-cooked treatments. On contrary, chemical pretreatment of the addition is shown to be effective removal of fibrous waxy barrier on tubers which increases the rate of drying.

3.3 Functional properties of palmyrah tuber flour

The functional properties of flour play an important role in the production of baked goods. The effect of different heat treatments on the functional properties of the heat-treated flour samples is represented in Table 3. The pH is an important physical parameter for estimating product quality. The results showed that there was no significant difference in the pH of flours made with different heat treatments. Treatment T2 (Raw oven dried at 60°C for 10hrs) was observed significantly highest pH level which indicated between 6.14 to 6.82. The lower pH of the palmyrah tuber flour could be an indication of storage stability against microbial contamination and a slight increase in pH, could be due to the slow fermentation process.

Bulk density is a measure of the heaviness and an indicator of the porosity of a sample flour. It is important to determine the packaging requirements, material handling, and wet processing application requirements in the food industry [17](Ocloo *et al.,*2010) and since flour with high bulk densities is used as a thickening agent in food products, flour from Palmyra tuber investigated could be used as thickeners are used.

The results shown boiled with 1% NaCl sundried for three days treated tuber flour and boiled with 1% NaCl oven-dried at 60°C for 10hrs treated tuber flour gave a higher bulk density meaning that this type of flour took up comparatively less space than another type of treated flours. The bulk densities of uncooked palmyrah tuber flour ranged from 0.43gcm^{-3} to 0.48gcm^{-3}. It is higher than that of commercially available uncooked tuber flour samples (0.40gcm^{-3}). On the other hand, the bulk densities of pre-cooked palmyrah tuber samples ranged from 0.62cm^{-3} to 0.70cm^{-3}. This result is significantly the same as that of the commercially available pre-cooked palmyrah tuber flour sample. However, the tapped densities were higher than their corresponding loose bulk densities. Low bulk-density flours are adequately used in complementary foods.

Ejiofor *et al.,* (2014) [18]described that water absorption capacity (WAC) is an important parameter affecting viscosity. Furthermore, the water absorption capacity is important for the volume and consistency of products and baking applications. Water absorption is strongly dependent on the crystalline properties of starch. High WAC is based on denatured protein [19].The denatured protein binds more water than native proteins through precooked methods such as boiling with 1% NaCl, boiling without NaCl, and steaming increases the WAC capacity more than others.

Table 3. Functional properties of heat-treated palmyrah tuber flour types

Treatments	pH	Bulk density	Tapped density	Water Absorption capacity	Oil absorption capacity	Foaming capacity	Foam stability	Solubility	Swelling power
T1	6.61±0.01^{d}	0.48±0.01^{d}	0.57±0.01^{f}	198±0.58^{h}	131±0.98^{e}	5.24±0.02^{g}	33.40±0.10^{b}	22.53±0.03^{g}	4.08±0.02^{j}
T2	6.82±0.01^{a}	0.43±0.01^{d}	0.54±0.01^{fg}	214±1.05^{g}	137±0.58^{c}	10.21±0.02^{a}	74.74±0.24^{a}	26.55±0.02^{d}	4.05±0.02^{j}
T3	6.44±0.01^{h}	0.45±0.01^{d}	0.53±0.01^{g}	181±1.13^{j}	151±1.53^{a}	5.02±0.02^{h}	75.00±0.56^{a}	11.09±0.01^{i}	4.23±0.02^{i}
T4	6.57±0.01^{c}	0.62±0.01^{b}	0.61±0.02^{e}	281±0.55^{c}	134±1.53^{d}	8.31±0.01^{b}	2.06±0.02^{d}	5.15±0.01^{l}	7.27±0.02^{b}
T5	6.34±0.00^{e}	0.63±0.01^{b}	0.69±0.01^{d}	303±1.50^{a}	145±0.58^{b}	6.24±0.01^{d}	2.08±0.03^{d}	13.45±0.01^{h}	4.57±0.02^{g}
T6	6.14±0.01^{j}	0.65±0.01^{b}	0.74±0.02^{bc}	272±1.78^{d}	111±0.58^{gh}	6.13±0.01^{e}	2.06±0.02^{d}	24.03±0.02^{f}	6.43±0.02^{c}
T7	6.32±0.01^{i}	0.70±0.01^{ab}	0.8±0.01^{a}	302±1.56^{a}	105±0.58^{j}	6.62±0.02^{c}	3.83±0.16^{c}	45.63±0.04^{a}	10.05±0.02^{a}
T8	6.77±0.02^{b}	0.68±0.01^{ab}	0.77±0.01^{ab}	261±1.50^{e}	112±1.00^{g}	4.02±0.02^{l}	3.89±0.03^{c}	5.23±0.03^{k}	4.44±0.03^{h}
T9	6.55±0.01^{f}	0.64±0.02^{b}	0.72±0.02^{cd}	297±0.92^{b}	126±0.58^{f}	8.32±0.02^{b}	4.16±0.02^{c}	26.33±0.02^{e}	5.64±0.03^{e}
T10	6.44±0.01^{h}	0.65±0.02^{b}	0.74±0.01^{bc}	302±1.53^{a}	109±0.58^{hi}	3.81±0.02^{j}	1.21±0.02^{e}	27.92±0.02^{b}	6.06±0.03^{d}
T11	6.67±0.01^{c}	0.64±0.01^{c}	0.62±0.01^{e}	269±1.50^{d}	125±0.58^{f}	3.72±0.02^{k}	2.17±0.02^{d}	27.43±0.03^{c}	5.17±0.02^{f}
T12	6.49±0.01^{g}	0.66±0.01^{b}	0.72±0.01^{c}	253±1.51^{f}	108±0.58^{j}	5.81±0.01^{f}	1.11±0.03^{e}	9.44±0.03^{j}	2.865±0.02^{k}

Table 4. Physio-chemical properties of heat-treated palmyrah tuber flour types

Treatments	Moisture %	Fat%	Ash%	Crude fiber%	Protein%	Carbohydrate %
T1	5.27±0.03^{g}	0.52±0.01^{cd}	2.13±0.02^{b}	2.63±0.01^{f}	1.75±0.01^{b}	85.26±0.03^{e}
T2	3.77±0.01^{i}	0.51±0.02^{d}	2.27±0.01^{a}	2.62±0.01^{f}	1.94±0.01^{a}	86.26±0.02^{c}
T3	3.89±0.02^{hi}	0.51±0.02^{d}	2.26±0.01^{a}	2.50±0.02^{g}	1.93±0.02^{a}	85.50±0.67^{d}
T4	10.6±0.04^{a}	0.71±0.01^{a}	2.03±0.01^{d}	3.25±0.02^{b}	1.64±0.02^{c}	83.74±0.03^{h}
T5	9.33±0.03^{b}	0.64±0.02^{b}	2.11±0.01^{b}	3.34±0.02^{a}	1.53±0.03^{d}	84.37±0.02^{g}
T6	8.52±0.02^{c}	0.53±0.01^{cd}	2.07±0.01^{c}	3.32±0.01^{a}	1.64±0.01^{c}	87.13±0.03^{b}
T7	5.6±0.03^{f}	0.67±0.01^{ab}	2.02±0.01^{d}	3.02±0.02^{d}	1.53±0.02^{d}	84.95±0.03^{f}
T8	3.43±0.02^{j}	0.53±0.02^{cd}	1.98±0.02^{e}	2.97±0.01^{e}	1.27±0.01^{g}	86.34±0.02^{c}
T9	3.93±0.04^{h}	0.56±0.02^{c}	2.05±0.01^{cd}	3.07±0.02^{c}	1.44±0.01^{e}	85.43±0.02^{d}
T10	6.12±0.04^{e}	0.63±0.02^{b}	1.98±0.01^{e}	3.07±0.02^{c}	1.33±0.02^{f}	85.22±0.02^{e}
T11	7.53±0.03^{d}	0.53±0.02^{cd}	1.96±0.01^{ef}	3.05±0.01^{cd}	1.70±0.02^{c}	85.03±0.02^{f}
T12	7.49±0.05^{d}	0.46±0.03^{e}	1.94±0.01^{f}	2.98±0.02^{e}	1.50±0.02^{e}	87.83±0.03^{a}

Each value in the table is represented as mean ± SD (n = 3), Means that do not share a letter are significantly different p<0.05

Based on the results showing that the water absorption capacity of the flour, T5 (Steam oven dried at 60^{0}C for 10hrs) was recorded as the highest (303%). However, there are no significant differences between T7 (Boiled with 1% NaCl sundried for three days), and T10 (Boiled without NaCl sundried for three days). The significantly lower water absorption capacity (181%) was found at T3 (Raw oven dried at 80^{0}C for 5hrs). Flours with high water absorption capacities can be recommended as humectants in food products such as cakes, bread, and pastries.

Oil absorption is also a measure of the amount of oil absorbed by the food during cooking. It is mainly due to the physical configuration of the oil through

capillary action on the hydrophobic components of the proteins [20]. The results showed significantly highest oil absorption capacity was observed in T3 (Raw oven dried at 80°C for 5hrs) flour followed by T5 (Steam oven dried at 60°C for 10hrs) and the lowest was recorded (105%) in T7 (Boiled with 1% NaCl sundried for three days). There was a significant difference ($p<0.05$) among all the heat-treated tuber flour samples. But commercially available pre-cooked and un-cooked palmyrah tuber flours exhibit comparably high oil absorption capacity may be that flour has high protein and flour made by a blend of different maturation stages and geographical locations of tubers.

Fats and oils are important factors in food systems as they contribute to the organoleptic properties (Mouthfeel, aroma, appearance, smoothness, and creaminess) of food products. Food ingredients with high oil absorption capacity are therefore suitable as lipid-based functional ingredients in food products such as cookies and crackers [21]. The heat-treated tuber flour especially un-cooked dried at 80°C for 5hrs, flours could therefore be used in these food products.

The eating quality is food is often related to the water-to-oil ratio in the swollen starch granules. Water and oil absorption capacities are important factors in baking applications as they build the structure and texture of baked goods. However, the processing of the raw materials affected the appearance, color, and texture and ultimately altered the sensory properties. Because of these properties, it provided a desirable taste and good mouth feel to the cookies, and biscuits and increases the moistness and softness of the baked products.

The foaming capacity of the heat-treated palmyrah tuber four samples ranged from 3.72% (Boiled without NaCl oven dried at 60°C for 10hrs) to 10.21% (Raw oven dried at 60°C for 10hrs) but it is less than that of the commercially available pre-cooked (16%) and un-cooked (75.67%) tuber samples. On the other hand, significantly lower foam stability was recorded in T12 (Boiled without NaCl oven dried at 80°C for 5hrs) (Table 3). Foaming capacity is important in assessing the suitability of incorporating a food ingredient into a food system. Food products such as cakes, cookies, ice cream, marshmallows, and bread require food ingredients with high foaming capacity [21]. However, food ingredients with low foaming capacity are suitably applied in biscuits, crackers, and cookies. Heat-treated flours could, therefore, be in the manufacture of biscuits, crackers, and cookies production because of their relatively low foaming capacity.

Depending on the result of the solubility of the treated flour types, the grades vary with the breakage of the starch granules. High solubility was obtained in T7 (Boiled with 1% NaCl sundried for three days) heat-treated flour due to the strong destruction and exposure of hydrophilic groups and the lowest in T4 (Steam sun-dried for three days). Data on solubility properties are very useful to determine optimal flour extraction conditions [22]. The high solubility could

also be attributed to the weak attractive forces between the molecules of the food material, leading to the greater dissolution of the food material in water [23].

Water absorption capacity is related to swelling power/capacity as both are functions of proteins and carbohydrates. Proteins and carbohydrates combine with water molecules through the formation of hydrophilic bonds [23]. In this study, the swelling power of the flour was significantly high at T7 (Boiled with 1% NaCl sundried for three days), lowest shown in T12 (Boiled without NaCl oven dried at 80°C for 10hrs) due to the presence of a large number of crystallites could be the reason for low swelling capacity which increases granular stability, thus reduces the extent of granular swelling. Food eating quality is connected with the retention of water-swollen starch granules. The high solubility of sun-dried flour could be due to increased hydrophilicity caused by an increase in the polar components (sugar, organic acids, and soluble proteins) as a result of different heat treatments method. Flours with high swelling capacity and water absorption capacity are recommended as functional ingredients in the production of viscous foods such as baked goods, dough, pasta, noodles, gravies, and soups [21]. Flour suitable for good quality functional ingredients has low solubility and high swelling capacity.

3.4 *Physicochemical properties*

The moisture content of the pre-cooked flour samples ranged from 3.43% to 10.66% with treatment T8 (Boiled with 1% NaCl oven dried at 60°C for 10hrs) flour recording the lowest moisture content while the treatment T4 (Steam sun-dried for three days) flour recorded the highest (Table 4). The moisture content of the uncooked tuber flour samples ranged from 3.77% to 5.27% with treatment T2 (Raw oven dried at 60°C for 10hrs) having significantly the lowest moisture content%. The moisture content of flour samples was lower than values reported in the literature for raw tuber (un-cooked) flour (10.8%) [5] and wheat flour (3.33%) [24]. Food products with high moisture content are susceptible to microbial attack and spoilage and therefore have a limited shelf life. Therefore, the low moisture content of the flour samples indicated their stability against microbial attack and possibly a longer shelf life.

According to the yield%, and moisture% results, the two heat treatments such as T2 (Raw oven dried at 60°C for 10hrs), and T8 (Boiled with 1% NaCl oven-dried at 60°C for 10hrs) are highly recommended for the industry due to the high yield% and the low moisture% in the large-scale production of palmyrah tuber flour. Fat contributes to the total energy content of a food. Therefore, its value is important to estimate the caloric value of a food. The fat content of the uncooked tuber flour samples ranged from 0.51% to 0.52% (Table 4). Significantly treatments T2 (Raw oven dried at 60°C for 10hrs), and T3 (Raw oven dried at 80°C for 7hrs had the similarly lowest fat%. On the other hand, the fat content of the pre-cooked tuber flour samples ranged from 0.46%

to 0.71%. Significantly treatment T4 (Steam sun-dried for three days) flour sample recorded the highest crude fat content (0.71%) whilst the treatments T6 (Steam oven dried at 80°C for 5hrs), T8 (Boiled with 1% NaCl oven dried at 60°C for 10hrs), T11 (Boiled without NaCl oven dried at 60°C for 10hrs) flour samples recorded the least fat content (0.53%). The palmyrah tuber normally contains 0.6% of crude fat [16]. However, the fat content of tuber flour was influenced by the pretreatment and different drying methods. When crude fat content was expressed on a dry weight attributed to the processing, there was a significant difference between the tuber and flour concerning their fat content and this could be attributed to the processing procedure, for example, leaching during treatments could reduce the fat content. Therefore, the oven-drying method is more suitable than the sun-drying method. The relatively lower fat content in palmyrah flour is desirable which makes the risk of oxidation reduced thus preventing the development of off-flavors resulting from rancidity. The fat contents of pre-cooked, uncooked flours were lower than that of soft wheat flour (1.33%). The low-fat content of the flour also makes it a suitable substitute for health-conscious and overweight people who want to reduce their calorie and fat intake.

Ash is the organic residue that remains after organic materials have been burned away [17]. The total ash content is directly proportional to the inorganic element content. A product's ash content is an indication of its mineral content, safety, and quality. The ash content of the uncooked palmyrah tuber flour ranged from treatment T1 (Raw sun-dried for 3 days) 2.13% to T2 (Raw oven dried at 60°C for 10hrs) 2.27%. On the other hand, the ash content of the pre-cooked palmyrah tuber flour ranged from treatment T12 (Boiled without NaCl oven dried at 80°C for 5hrs) 1.94% to T5 (Steam oven dried at 60°C for 10hrs) 2.11%. According to the result, the steam cooking method reduces the inorganic elements compared to other cooking methods such as boiled with 1% NaCl, boiled without NaCl, and un-cooked). The reduction in total ash may be due to mineral compound leaching and water absorption during boiling and steaming [25]. The inorganic content of the tuber can vary depending on the time at which the tuber was harvested and its geographic location. This can lead to a discrepancy in the ash content of commercially available palmyrah tuber flour.

The uncooked palmyrah tuber flour samples had a crude fiber content of 2.50% whilst the pre-cooked tuber flour had the highest value of 3.34% (Table 4) therefore, treated palmyrah tuber flour produced a good health effect. The flour samples, however, had a higher fiber content in comparison to the maize flour (0.20%-0.85%), banana flour (0.11-3.50%), soybean flour (0.88%), millet flour (0.27%-2.70%), wheat flour (0.36%-0.82%) and cassava flour (1.48%-1.54%) [23].Dietary fiber is good for preventing or controlling diabetes, cardiovascular diseases, obesity, and cholesterol levels in the body by interfering with its absorption. The palmyrah palm flour

samples may meet this recommendation when consumed appropriately. Dietary fiber also gives a feeling of satiety; therefore, the flour could be applied in the production of lower glycemic index foods for Type-II diabetics.

As the crude protein, the palmyrah tuber and tuber flour recorded 8.54% [16]and 3.1% [5] respectively, Palmyrah tuber flour protein content was lower than that of wheat flour (11.8%), corn (7.5%) and rice (7.0%). In this study, the un-cooked tubers dried in an oven at 60°C for 10hrs shown 1.94% (T2) of crude protein, and un-cooked tubers dried in an oven at 85°C for 7hrs shown 1.93% (T3) of crude protein designated that no significant changes had because of changing the temperature or drying period. The protein content of the pre-cooked tuber flour samples ranged from 1.27% to 1.70%. However, the crude protein for commercially available un-boiled tuber flour was recorded as 3.19% and pre-cooked tuber flour was recorded as 1.47%. The crude protein of the different heat treated palmyrah tuber flour studied was lower than the values described in the literature for wheat flour (10.23%-14.70%), and millet flour (6.44%). However, in the treated tuber flour it was observed the reduction of protein and it could have been due to protein denature throughout the different pretreatments such as boiling with 1% NaCl, boiling without NaCl, and steaming. [25] Fekadu *et al.,* (2013) stated that the reduction of crude protein during boiling may be attributed to the leaching and denaturing of protein caused by boiling, but in here boiling with NaCl and steaming also decrease the protein content. Thus, the low protein content of Palmyra tuber four may be a result due to the climatic aspects and also soil type.

Carbohydrates provide quick energy and support in fat metabolism. Total carbohydrates were affected by other macronutrients and the moisture content of the flour. The total carbohydrates of the uncooked flour samples ranged from 85.26% to 86.26% (Table 2) higher than the commercially available sample (77.1%) [5]. On the other hand, the total carbohydrates of the pre-cooked flour samples were intermediate between the flour samples from treatment T12 (Boiled without NaCl oven dried at 80°C for 5hrs) flour samples recorded the highest value at 87.83% while the treatment T5 (Steam oven dried at 60°C for 10hrs) flour recorded the lowest available carbohydrate value 83.74%. The heat-treated palmyrah tuber flour was better than banana flour (76.68%), maize flour (71.87%-85.64%), cassava flour (75.50%-75.90%), and millet flour (89.19%) but higher than the available carbohydrate content of soya bean flour (26.14%) and cowpea flour (57.35%) [23]. The relatively higher carbohydrate content indicates that the heat-treated flour samples are a good source of energy for the body. Nutritionists could therefore recommend the use of flour in high-energy foods. Carbohydrates also play an important role in the functional properties of flour samples by association with water molecules through hydrogen bonding, resulting in water absorption and swelling power

[21]. Therefore, the flours could serve as thickeners in food products.

IV. CONCLUSION

The Palmyrah palm (*Borassus flabellifer*) tuber flour had considerably high amounts of crude fiber and carbohydrates. The high fiber content of palmyrah tuber flour can be discovered in the production of low glycemic index, hypolipidemic and prebiotic foods. The moderately high swelling power, water, and oil absorption capacities also designate the palmyrah tuber flour could be broadly applied in the pastry industry as a binder, stabilizer, thickener, humectant, and flavor retaining agent. The different heat-treated tuber flour can be applied in the production of bread, cookies, cakes, pasta, pastries, noodles, gravies, sauces, and soup mix due to its high swelling power and water absorption capacity. The low foaming capacity also makes heat-treated tuber flour a potential ingredient in the biscuit industry as well as in the production of crackers.

Among these selected five districts, Mannar district has the highest yield ratio and low moisture content. Comparatively the use of boiled with NaCl and drying at 60°C heat treatment and raw tuber drying at 60°C heat treatments existing the best option considering the cost, drying temperature, and time on the yield and quality of tuber flour.

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EVALUATION OF THE EFFICACY OF BOTANICAL INSECTICIDE FORMULATIONS FOR THE MANAGEMENT OF BANANA MEALY BUG *Pseudococcus elisae*

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Abstract*:* Various pesticides are available to help farmers overcome yield losses in banana production. The existence of a waxy covering around mealybug's body, on the other hand, makes them extremely difficult to control. The waxy coating, high reproduction rate, symbiosis with ants and a wide range of hosts are all elements that contribute to this pest's survival. Most farmers use synthetic insecticides to control the banana mealy bug, but there is a pressing need to discover other methods due to rising environmental and economic concerns. To tackle this threat, plant-derivative botanical pesticides or bio-rational can be employed as cost-effective, readily available, and environmentally feasible alternatives. Consequently, the study

aimed to formulate and evaluate the five alternative bio-rational formulations using available plant essential oils in laboratory and field settings. Descriptive statistics, one-way ANOVA and Tukey's posthoc test were utilized to analyse the data. The results revealed that mealybug populations can be reduced with 15% dosage bio-rational formulations. The results also revealed that all three treatments; T3 (menthol: castor oil: clove oil: liquid soap (5:5:1:1)), T2 (castor oil: clove oil: liquid soap (5:1:1)) and T1 (neem oil: castor oil: liquid soap (5:5:1) are statistically significant in reducing mealybugs in banana plants compared to the control where any bio-rational formulations have not used.

Keywords: **Banana Cultivation, Banana Mealybug, Mealybug control, Bio rational formulation**

I. INTRODUCTION

Bananas are considered one of the world's most popular commercial fruit crops, grown primarily in Asia, Africa, and the tropics and subtropics. Likewise, bananas are the most popular and widespread fruit in Sri Lanka, surpassing all other crops [1]. Commercial banana plantations are generally located in the dry zone of Sri Lanka. Based on Department of Agriculture, Sri Lanka in 2017, Sri Lanka produced around 62,549 tons of bananas on a land area of 47,096 acres. Many popular varieties of bananas

are grown in Sri Lanka, which are in great demand around the world. Of the millions of banana varieties available worldwide, the "Cavendish" banana (*Musa Accumulate*) is the most popular and commercially grown for the Sri Lankan export market. In 2002 Central Bank of Sri Lanka indicted that banana cultivation is influenced by several biotic and abiotic factors and such agro-industrial operations face significant challenges such as pests and diseases. Banana mealy bug (*Pseudococcus elisae*) is of serious concern among the pests of banana cultivation and has significant economic and aesthetic implications for banana cultivation and the banana industry [2]. These species have been reported to cause the most severe damage by sucking the cell sap from fruits, leaves and young shoots [3].

Farmers rely more on the chemical pesticide to control pest infestations around the world and mealybug infestations in banana plantations controlled with synthetic pesticides such as Dursban which contains the active ingredient chlorpyrifos but direct application of Dursban to banana fruit leads to huge amounts of edible pesticide residues [4]. In addition, the use of chemical pesticides has many environmental, health and residue effects, which are urgently encouraged to develop environmentally friendly practical solutions. A few studies have reported the use of natural predators, including predatory insects, naturally occurring parasitoid wasps and other predators, that can be used to control the banana mealybug [4,5]. For mealybugs and other pests, experts around the world are striving to develop and mainstream plant-based insecticides, often known as phyto pesticides, botanical pesticides, bio pesticides, or natural pesticides, by limiting use of harmful synthetic insecticides.

Moreover, synthetic chemicals have negative impacts on health and the environment, such as the extinction of natural predators and a decrease in biodiversity, raising environmental concerns. In addition, health threats with side effects and new chronic diseases are widespread among farming communities [6]. In addition, the ban and restricted use of highly hazardous pesticides in Sri Lanka has created the problem of a lack of effective synthetic insecticides to control the pest. Accordingly, finding efficient alternatives to control banana mealybug with minimal impact on the environment and human health is a serious concern. Therefore, the goal of this research is to find an effective bio-rational or botanical insecticide formulation with an effective dosage to control the banana mealybug under field conditions.

II. METERIALS AND METHODS

A. Study Site

The present experiment was conducted at the Dole Lanka (Pvt.) Ltd, Kuda Oya farm in Monaragala District, Sri Lanka where environmental conditions favour the growth of plantains and bananas. The Kuda Oya has a tropical climate. The farm at an elevation of (101.0 m) mean sea level, this farm has typical dry zone characteristics (low country

intermediate zone) (latitude 6.5303768, longitude 81.1505254).

B. Mealybug Collection and Culture

A colony of mealybugs was collected from the infected banana plantations (var. Cavendish) and maintained in the laboratory by providing the fresh green banana fruits until use for the laboratory bioassay. The growth conditions for the culture were 28 ± 2°C temperature with 60 ± 5% relative humidity.

C. Preparation of Plant Extracts

The different bio-rational formulations (15 treatments) with 3 replicates were prepared using commercially available neem oil, castor oil, clove oil and citronella oil in five different concentrations as 1%, 2%, 5%, 10% and 15% with the following formulations.

T1(1%, 2%, 5%, 10%, 15%) = neem oil: castor oil: liquid soap (5:5:1)
T2 (1%, 2%, 5%, 10%, 15%) = castor oil: clove oil: liquid soap (5:1:1)
T3 (1%, 2%, 5%, 10%, 15%) = menthol: castor oil: clove oil: liquid soap (5:5:1:1)

D. Assessment of Botanicals in Laboratory

A laboratory bioassay was performed using the prepared formulation and 30 adult mealybugs were introduced into each bioassay unit, which consisted of a petri dish with fresh green banana fruit, and the prepared formulation was sprayed onto bioassay rigs (each received 3ml of treatment solution) and the observation recorded at 24, 48, 72 and 96 hours after spraying. Each experiment with its replicates was repeated five times.

E. Field Experiment

The present experiment was carried out in a planted banana farm which was about 500m away from other plantain and banana fields with well-drained soil and level topography. The experiment was carried out for 6 months (October 2020 to March 2021 – usually involving the dry season with higher infestation and part of the rainy season).

The field experimental setup was a randomized complete block design with four treatments comprising the most effective doses from the laboratory experiments and replicated ten times each. Evenly grown banana cultivation blocks with a minimum distance of 500 m from each other were selected. Each plant was tagged with red tape for easy identification, and prominently labelled papers were placed in clear plastic to prevent rain damage. The plant emulsion for field application was prepared by adding the most effective dose of dilute with water (1:20) to the respective plant powder in 100ml emulsifier concentrate to produce the respective plant emulsions in the treatments (T).

T1 (15%) = neem oil: castor oil: liquid soap (5:5:1)
T2 (15%) = castor oil: clove oil: liquid soap (5:1:1)
T3 (15%) = menthol: castor oil: clove oil: liquid soap (5:5:1:1)
T4 = Untreated control (not spraying)

One week prior to application of the treatments, the pseudo stems of the banana plants were examined for the presence of mealybugs. Plants were

treated with the appropriate treatments using a knapsack sprayer one week after observation. The pseudo stems of each plant (1 meter from the ground) received about 1000 ml of each treatment solution, which was treated once a month up to three months.

F. Data Collection

One week prior to application of the treatments, the selected unit sample areas of the pseudo stems were counted and recorded as treatment compliant and apply the treatments. Then, using a hand lens, the number of mealybugs on the pseudo stems (1 m above the ground and 60 cm below the conger leaves) of the plants was inspected and counted 24 hours, 48 hours, 72 hours and 96 hours after spraying the formulations. Density was determined by counting the number of mealybugs in a measured 1m x 1m area through the sampled area counts and multiplied by the total sampled area as follows.

Mealy bug population Density = Density in sampled area x selected total area, selected total area = 2πr2 + h (where π = 22/7, r = radius, h = height) 2πr2 + h (2πr) is the formula of a cylinder (pseudo stem considered as a cylinder). The height and circumference of the selected area for each plant were measured with a tape.

G. Statistical Analysis

Statistical analysis was performed using SPSS software version 25. Means, variance and standard deviations within the treatments were calculated using descriptive statistics. According to the Kolmogorov-Smirnov ($p<0.05$) and Shapiro-Wilk ($p<0.05$) normality test data, the data were not normally distributed. Therefore, an Lg10 transformation was applied to the data to turn them into normal values. Then the different means were compared by One-way Analysis of Variance (ANOVA) while post hoc (Tukey's pair-wise) comparison test at $P = 0.05$ was used to determine the significant differences among the calculated means of the various treatments.

III. RESULTS AND DISCUSSION

A. Effects of botanicals on mealybug mortality and repulsion in the laboratory

Present study carried out to evaluate the treatments against banana mealybugs revealed that, among the all concentration of treatments tested (1%, 2%, 5%, 10% and 15%) all treatments at 15% were statistically on par and found significant over all the treatments ($F = 5.03$, $P. = 0.029$). Also, the results of the laboratory bioassay (15% concentration) indicated that there was a significant percentage mortality of mealybugs after 24hrs ($F=4.54$, $p= 0.014$) and according to Tukey's test (see Table 1) there was a significant difference between treatments and control but there was no significant difference when the different treatments were compared. Figure 1 illustrates the mealybug counts in laboratory bioassay before and after treatment for 15% concentration. As illustrated mealybugs count was same in control for both pre and post treatment.

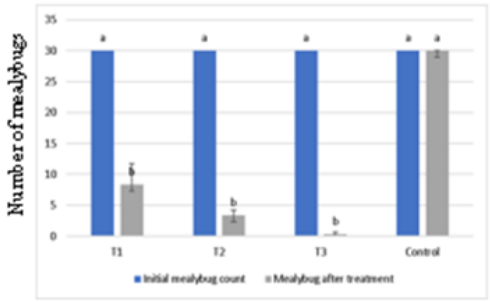

Figure 1. Number of live mealybugs in the laboratory bioassay

(The error bars indicated the standard error of mean. The same letter is not significantly different Tukey's post hoc test at p = 0.05, n = 4)

B. Effects of botanicals on mealybugs in the field

Figure 2 illustrates the mealybug count in pre and post treatments in the field study. In field there was no significant difference in mealybug counts in pre survey (F = 2.98, p = 0.9235). Figure 3 illustrates the mealybug density/counts/ population in pre and post field survey. Further considering the pre counts also indicated that there were no significant differences among treatments (F = 3.86, p = 0.768). As illustrated in the figure the live mealybug counts were reducing after the application of treatments and the reduction was increasing with the time.

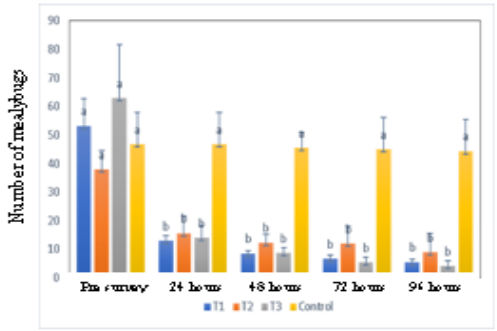

Figure 2. Mean count of Mealybugs in the field plants

(The error bars indicated the standard error of mean. The same letter is not significantly different Tukey's post hoc test at p = 0.05, n = 4)

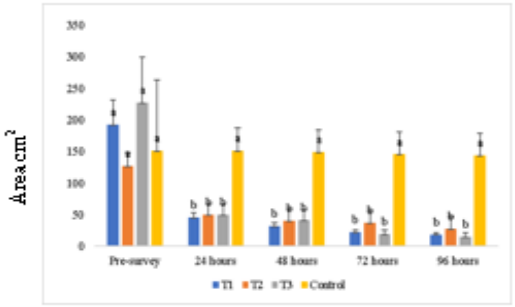

Figure 3. Distribution of mealybugs in field plants

(Error bars indicate the standard error of the mean. The same letters are not significantly different in Tukey's post hoc test at p=0.05, n = 4)

Figure 4 further illustrated the mortality of mealybug with treatments and time in field. As it illustrates mortality was increased after the application of treatments. The highest mortality reported in the plants with application of

T3 compared to other treatments followed by T2 and T1 respectively. Further it was indicated that the mortality rate increases with the time after spraying botanicals in the field. According to the one-way ANOVA and Tukey's test in field after the 24hrs, 48hrs, 72hrs and 96 hours of treatment application there were significant difference in the mean mealybug counts with the control at 5% significant level (see Table 1) but there was no significant difference when the deference among the treatments compared and this was true for all observation time period. Also in control mealybug count is increasing with time since the mealybugs attracted by banana with time because of the ripening of fruits.

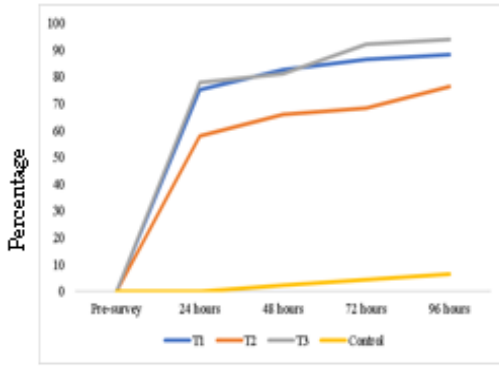

Figure 4. Mortality of mealybugs with treatments and time in field

All botanical formulations used in this study differ in terms of mortality rates. This indicates that botanical pesticides are primarily contact pesticides. This is due to the fact that when these insects come into contact with plant extracts, they become poisoned, weakened and die. The results obtained in this study are consistent with other studies showing that the botanicals used in the study can reduce the population of various types of pests [4] and that EOs have shown significant effects in controlling mealybug species [7].

Plant essential oils (EOs) are volatile organic compounds with strong aromatic components that are extracted from specific plant parts such as seeds, petioles, or peel, depending on the plant species [8]. The presence of multiple bioactive ingredients in plant EOs are beneficial and because they are lipophilic, they can penetrate the cuticle of insects, leading to dehydration of the insects [9]. Numerous neem compounds have deleterious effects on pests, altering their behaviour, physiology and developmental stages by affecting the endocrine system, particularly in the case of ecdysteroids, resulting in growth stunting, deformities and insect mortality [10]. Castor bean is a wild plant in large ecological areas of the world. The plant contains 90% ricin, 4% linoleic acid and 3% oleic, 1% stearic and less than 1% linolenic fatty acids. It causes insect suffocation by inducing lipid membrane disruption [11]. Soaps are affordable and effective against many soft-bodied pests such as aphids, soft scales, psyllium, whiteflies, mealybugs, thrips and spider mites. Soap sprays help break down the wax and expose the insects' outer coverings, causing them to lose water [12]. In this context, the highly bioactive organic compounds from plants offer an opportunity for the development of a useful and sustainable strategy to protect banana plantations from mealybugs.

Our results are consistent with the findings of Mossa [13] that camphor, citronella, clove, menthol and geraniol were used for this study and induced the repellence against several insects, and is also consistent with the findings of Butnariu and Sarac [14] compatible. that volatile liquids are extraordinarily complex chemical substances that work with great potency and precision. Furthermore, these plant materials are relatively abundant in the study area and the ease of preparing the plant emulsions makes them a viable management strategy for inclusion in the local IPM strategy for managing mealybug infestations in banana and plantain fields.

Table 1. Live mealybugs with different treatments before and after the treatment applications

Treatment	Live mealy bugs on the plants (Mean (±) SE)/unit area				
	Pre treatment	24hrs After treatment	48hrsAfter treatment	72 hrs After treatment	96 hrs After treatment
T1	53.2±9.4 a	45.2±7.3 b	31.9±5.0 b	22.6±3.7 b	18.7±2.7 b
T2	38.0±6.6 a	50.0±17.7 b	40.2±18.6 b	37.4±18.6 b	27.5±19.5 b
T3	62.9±18.8 a	49.9±16.4 b	41.6±15.2 b	19.0±6.7 b	15.0±6.6 b
control	46.8±11.1 a	151.3±35.5 a	148.7±35.1 a	145.4±35.1 a	143.5±35.0 a
F(Df1,Df2)	4.108	4.696	24.828	7.456	6.956
P	0.013	0.007	0.003	0.001	0.001

The values are means ± Standard error (SE); Within a column, means followed by the same letter are not significantly different Tukey's posthoc test at p = 0.05, n = 4

IV. CONCLUSION

The main aim of this study is to manage the mealybug population in banana cultivation in an environmentally friendly way. Recently, essential oils have been used to repel banana mealybug due to their low mammalian toxicity and rapid environmental degradation compared to traditional pesticides. Biological control of mealybugs on cotton crops can show promising results. The results obtained from laboratory and field studies show that botanical pesticides can reduce the population of mealybugs per unit area.

All three treatments, T1 (Neem Oil: Castor Oil: Liquid Soap (5:5:1)), T2 (Castor Oil: Clove Oil: Liquid Soap (5:1:1)), and T3 (Menthol: Castor Oil: Clove). Oil: Liquid soap (5:5:1:1) may be recommended for banana mealybug control after large-scale field evaluation.

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EFFECTS OF SELECTED PLANT GROWTH PROMOTING RHIZOBACTERIAL STRAINS ON GROWTH PERFORMANCE OF COWPEA (*Vigna unguiculata* L.)

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Abstract: Plant Growth-Promoting Rhizobacteria (PGPR) are a diverse group of soil bacteria that inhabits the rhizosphere and directly or indirectly induce plant growth and development. The present study was designed to investigate the plant growth performance properties of rhizobacteria (*Pseudomonas fluorescens*, *Bacillus megaterium, and Azotobacter vinelandi)* and identify the unique function of PGPR in the growth of cowpea (*Vigna unguiculata* L.). Five treatments were performed with 6 replicates. Accordingly, each treatment was applied to 48 sterilized seeds. Except for control, sterilized seeds were added with a different volumetric combination of bacterial strains and 25 ml of the seed coating solution. Simultaneously, seeds were planted in pots and kept under greenhouse conditions. Growth parameters namely, seed germination percentage, plant height, fresh weight, dry weight and root length were recorded. Plant height was recorded at 1st, 2nd, and 4th week after planting while the rest of the parameters were recorded at 4th week. One way ANOVA was performed using SPSS (Version 25). Results revealed that, germination was remarkably induced by *Pseudomonas fluorescens* and the germination percentage of each treatment significantly differed (p =0.036) from the control. All the growth parameters tested showed a significant difference among all treatments. Anyhow, based on the ratio of bacterial strains used, the change observed in plant growth parameter differed significantly compared to control. Plant height at 1st and 4th week has been highly induced by *Azotobacter vinelandi* and *Pseudomonas fluorescens* respectively and it was significantly different compared to control. The treatment dominated with *Azotobacter vinelandi* provided the highest biomass while it was significant to the control. Root elongation also significant among treatments and highly induced by abundant *Bacillus megaterium* in the solution.

Keywords: **Cowpea, Growth parameters, Rhizobacteria, Plant growth, Seed coating.**

I. INTRODUCTION

A heterogeneous group of bacteria termed plant growth promoting rhizobacteria (PGPR) [1] can be found in the rhizosphere i.e. on the surfaces of roots and in the immediate vicinity of roots [2]. Free-living soil organisms that thrive in the rhizosphere, called

rhizobacteria, aggressively invade plant roots and promote plant growth [3].

PGPR can either directly or indirectly increase the extent or quality of plant development [4]. In the early stages when [5] used *Pseudomonas fluorescent* spp. as a growth promoter capable of resisting plant diseases, the concept of PGPR was evolved. Since then, the term was used to refer to any rhizobacteria that can directly promote plant development [6].

Pseudomonas fluorescens, *Bacillus megaterium, Bacillus subtilis*, and *Bacillus cereus* are some examples for PGPR [7] and their metabolites may play an important role in controlling plant diseases such as root rot and wilt while improving plant growth parameters [8]. *Bacillus* species and *P. fluorescens* are root colonizers that can induce plant defensive resistance [4]. It has recently been used to denote a variety of rhizobacteria including *Alcaligenes, Pseudomonas, Azospirillum, Bacillus, Klebsiella, Azotobacter, Enterobacter, Burkholderia, Arthrobacter,* and *Serratia*, which promote plant development through various processes [9].

PGPR have a unique function in the plan t's ability to resist disease, takeup more nutrients, produce roots and shoots, pr omote seed germination, and be more r esilient to environmental stresses [10]. It turns out that these organisms, support for a variety of function, including enhanced nitrogen fixation through nodule formation, solubilization of phosphates, production of phytohormones such as gibberellins and siderophores as well as indoleacetic acid, and acting as low molecular weight agents regulating plant growth and development [11].

However, [12, 13] claim that there are environmental factors such as salt and drought stress that adversely affect the ability of bacteria to promote plant growth (PGP) and phosphate solubilization. Despite the existence of PGPR in the rhizosphere, additional outer coating of seeds with the PGPR as a tool to deliver beneficial microbes to agricultural crops can enhance plant growth and development [14] since seeds are the basic factor of every plant with sexual reproduction. Application of various seed treatments can improve seed health and performance, ultimately leading to better productivity [15]

Seed coating is one of the viability enhancing seed treatments and has been mainly applied to crops and vegetables [16]. Seed coating is the process of applying exogenous material to the surface of the seed and represents a cost-effective and environmentally friendly approach to crop protection [17] increase in germination percentage and plant establishment.

Seed coating with PGPR improves seed weight, size, appearance, and also seed protection from phytopathogens [18]. Due to the presence of a seed coating, improved nodulation in legumes improves seed germination and protects seeds and seedlings from pathogens and insects [14]. Seed coating has the ability to absorb pesticides and fungicides and, protect the high value of the seed while protecting against insect or plant pathogens. Therefore, the

coating process minimizes pesticides and is an environmentally-friendly method [19].

However, the PGPR seed coating process has been applied to many crops to study their various performances, including plant growth and development that showed positive results. For example, the studies performed for many commercial crops such as maize [20], banana and cotton [21], [22], cucumber[23] rice [25] have shown improved growth and productivity. However, use of PGPR is rarely applied to cowpea crop compared to other fruits and vegetable crops. Therefore to fill the gap in the literature, this study was designed to evaluate the effect of PGPR strains on the growth parameters (plant height, root length, fresh and dry weight) of cowpea plant (*Vigna unguiculata*) when PGPR applied before planting.

The results of this study will be helpful for further studies on the physiology and biology of the cowpea plant and how to create a sustainable environment.

II. Methodology and Experimental design

The experiment was conducted at the laboratory facilities of IAMS Private (Ltd.), Alawwatta, Wariyagoda, Alawwa, Kurunegala District. Cowpea seeds of Dawala Variety was been selected based on being free from contaminants, weed seeds, insects, and foreign matter. Three bacterial strains were selected, namely *A. vinelandi, B. megaterium, and P. fluorescence* for seed coating treatment prior to planting. Stock cultures of each bacteria strain were used to make subcultures of the required quantity.

A. *Subculturing of bacteria*:

1) Nutrient agar preparation:

The nutrient agar solution was prepared according to the standard method of preparation [25] while 2.1 grams of nutrient agar powder (consisting of 0.5% peptone, 0.3% beef extract/yeast extract, 1.5% agar, and 0.5% NaCl) was dissolved in 90 ml of distilled water. The mixture was heated well with stirring to dissolve all components and the dissolved mixture was autoclaved at 121℃ for 15 minutes. Thereafter, the mixture was allowed to cool and poured into petri dishes (30 ml each) by placing the plates on the sterile surface (Laminar air flow) until the agar solidified. Subsequently, stock cultures of each bacteria strain were inoculated into the nutrient agar plate using the streak plate method with a sterilized loop. Once the inoculation was done the plates was incubated at a temperature of 28°C for 2-4 days.

2) Preparation of nutrient broth and introduction of bacteria strains:

Here, 0.6 grams of nutrient broth powder was dissolved in 300 ml of distilled water, poured into three equal volume (100 ml) of labelled Erlenmeyer flasks (labelled with bacterial strains), and sterilized [26] by autoclaving at 121°C for 15 minutes.

Thereafter, an isolated single colony was picked from incubated Petri dishes and placed in each Erlenmeyer flask with 100 ml of prepared nutrient broth at

room temperature. Thereafter, different volumetric combination of bacterial strains were prepared as shown in Table 1, hence 5 treatments were ordered for this experiment.

Table 01. Different treatments with the mixing ratio of bacteria

Treatment	*Pseudomonas fluorescence*	*Azotobacter vinelandi*	*Bacillus megaterium*	Distilled water (ml)	Ratio
T1	5 ml	5 ml	5 ml	15 ml	1:1:1
T2	10 ml	5 ml	5 ml	5 ml	2:1:1
T3	5 ml	5 ml	10 ml	5 ml	1:1:2
T4	5 ml	10 ml	5 ml	5 ml	1:2:1
T5	0 ml	0 ml	0 ml	25 ml	0:0:0

The seed coating material was prepared according to [27]. Here, 150 ml of distilled water was boiled (100℃) in a magnetic stirrer and 1g of carboxymethyl cellulose (seed coating material) and 1g of gelatin were added and dissolved. 25ml of the dissolved mixture was added per bottle.

B. Sample preparation

Surface sterilization was done for cowpea seeds [28] by dipping into water sterilized with 70% ethanol for 5 min. After sterilization, for the bacterial treatment, each 60 cowpea seed and a bacterial treatment solution (with different combination of bacteria strains) was added into 25ml of seed coating solution as mentioned above. Subsequently, coated seeds with the bacterial solution and coating solution were kept in laminar airflow for 20min.

For the control (T5), 60 sterilized seeds were directly planted without any introduction of bacterial and seed coating solution.

C. Pot experiments

Pot experiments on the cowpea plant were set up with five formulations of seed treatment including 4 bacterial treatments T1, T2, T3, T4, and a bacteria-free treatment (T5). Each treatment was replicated six times (6 pots were used). Each pot was filled with sterilized soil and 8 seeds were sown. Altogether each treatment was applied to 48 cowpea seeds planted and pots were placed under greenhouse conditions.

D. Growth parameters

The germination rate (Number of germinated seeds/ Number of seeds planted) was determined [29] after 7 days of seed sowing in pots. Plant height was recorded from each plant in 1WAP, 2WAP and, the 4WAP. Thereafter at 4WAP, plant growth parameters root length, plant fresh weight, and dry weight (including root) [30] were determined to evaluate the growth parameters of each treatment.

E. Experimental design & statistical analysis:

Treatments were arranged in complete randomized design and statistical analysis was performed by using a SPSS (version 25). The results of all the measured parameters were given as the arithmetic mean of 5 independent measurements. Then, one-way analyses of variance (ANOVA) and significant differences between individual means were ascertained by Turkey's post hoc test. The statistical significance in all analyses was defined at $p<0.05$.

III. RESULTS AND DISCUSSION

3.1 Effects of PGPR on growth and development of cowpea

Table 02: Germination % of cowpea seeds with different treatments at 1WAP

Treatments	Mean ± SE	Germination (%)
T1	6.3 ± 0.5	79.2 b
T2	6.3 ± 0.3	79.2 b
T3	6.2 ± 0.4	77.1 a
T4	5.5 ± 1.0	75.0 a
T5 (Control)	4.5 ± 1.0	72.0 a

*(N = 48 (8*6); p value < 0.05)*

.Germination percentage of cowpea seeds significantly differ (p value = 0.021) with the different combination of bacterial treatments, whereas T1 and T2 showed significant germination percentage (*p* value = 0.036) compared to control.

Table 03: Growth parameters of cowpea seedlings under different treatments

Treatment	Plant height after planting (cm)			Fresh weight per plant (g/plant) - 4WAP	Dry weight (g/plant) 4WAP	Root length (cm/plant) 4WAP
	1WAP	2WAP	4WAP			
T1	10.2 ± 0.8 ^{ab}	17.0 ± 0.3^{a}	26.2 ± 0.9 ^{b}	15.1 ± 2.8 ^{b}	8.5 ± 0.8 ^{a}	5.6 ± 0.5 ^{a}
T2	10.6 ± 0.4 ^{ab}	15.9 ± 0.7 ^{ab}	29.4 ± 1.5 ^{a}	12.7 ± 3.0^{b}	9.2 ± 0.4 ^{a}	5.5 ± 0.2 ^{b}
T3	10.7 ± 1.2 ^{ab}	16.2 ± 1.5 ^{ab}	26.8 ± 1.2 ^{b}	15.0 ± 4.3^{b}	7.5 ± 1.0^{a}	6.2 ± 0.4 ^{a}
T4	11.2 ± 0.9 ^{a}	16.6 ± 1.1 ^{ab}	27.1 ± 0.5 ^{b}	20.5 ± 3.6^{a}	8.4 ± 0.8^{a}	5.8 ± 0.3^{a}
T5 (Control)	7.8 ± 0.3 ^{b}	13.1±1.6 ^{b}	22.7±1.4	5.1 ± 0.6	3.9 ± 0.7 ^{b}	4.1 ± 0.2^{b}
F value (df)	2.98 (4, 25)	2.98 (4, 25)	4.32 (4, 25)	3.10 (4, 25)	7.01 (4, 25)	5.71 (4,25)
P value	.039	.038	.009	0.026	0.001	.002

The values in each cell for the different treatments are mean ± standard error. Different letters on the values indicates the significant difference between the treatments ($p \leq 0.05$) ($n = 5$).

The measurements indicate that treatments applied increased the overall plant growth parameters; plant height, dry and fresh weight, and root length compared to the control and the treatments show significant differences (P<0.050) in each plant growth parameter recorded (Table 3).

Plant height recorded at 1WAP has significant differences between all treatments with the control while T4 shows significant differences compared to the control. The lowest and highest plant heights at 1WAP were 7.8 ± 0.3 cm (control) and 11.2 ± 0.9 cm (T4), respectively. The tallest plant height attained by from T4 had the bacteria strain combination ratio of (1:2:1) where *Azotobacter vinelandi* takes the highest proportion of than other two bacteria strains.

Azotobacter species' diversity and usefulness of *Azotobacter vinelandi* have been thoroughly recorded by various ecosystems for their ability to promote plant growth for sustainable agriculture [31]. According to [32], *Azotobacter vinelandi* boosts a seed's ability to by 20–30% by developing chemicals that promote plant growth. These compounds reduce chemical nitrogen and phosphorus requirements by 25% [33], and increasing plant growth [34]. The formation and release of secondary metabolites [35], such as plant growth regulators, or facilitating of uptake of specific nutrients from the root environment are two examples of how PGPR can directly promote plant growth, particularly the Azotobactor [36].

Furthermore, the study was conducted by [37] found that the combination of Azotobacter and Azospirillum bacteria increased plant growth traits and reduced the application of nitrogen fertilizer by 50% when applied together to sunflower plants at various levels of nitrogen. Similarly, the use of Azotobacter can reduce the need for nitrogen fertilizer. Bacterial strains isolated from the plant rhizosphere (PGPR) produce plant growth-promoting phytohormones that lead to shoot and root length elongation and seed germination of several agricultural crops [38].

The plant height recorded with 2WAP differs significantly ($p<0.05$) between all the different treatments applied including the control. In any case, the treatment prepared with similar proportions of bacteria strains (T1) shows the highest plant height (17.0 ± 0.3 cm) and is significant compared to control, which has the lowest plant height at 2WAP (13.1 ± 0.7 cm). Likewise, the plant height recorded at 4WAP show significant differences in all treatments, while plant height in T2 shows the highest proportion of *P. fluorescence* recording the highest plant height (29.4 ± 1.5 cm) compared to other treatments and it is significant compared to control (22.8 ± 1.4 cm). *P.*

fluorescence is important for phosphate solubilisation.

Phosphate is highly available in the soil but it is in the form of insoluble. Where *P. fluorescence* spp converts an insoluble form of phosphate into a soluble form. Then these bacteria cause the availability of phosphate to the plant, thereby increasing plant growth and development [39].

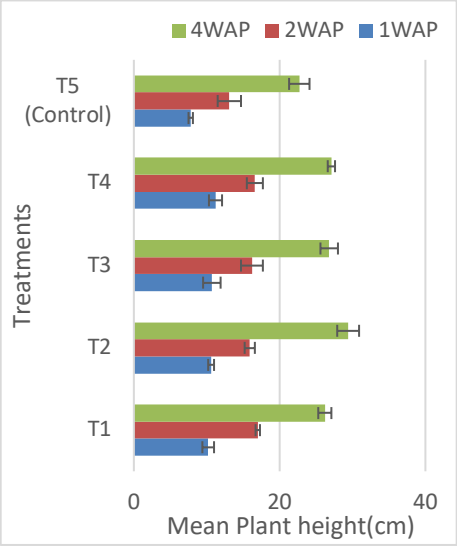

Figure 1: Progress of plant height under different bacteria combinations

Therefore, as shown in Figure 1, the plant height recorded at 1WAP, 2WAP and 4WAP increased significantly despite the different combinations of bacterial strains used for seed coating. Compared to the control treatment, all other treatments consisting of bacteria show a significant increase in plant height recorded for three intervals.

Root length, dry weight, and fresh weight were recorded at 4WAP as figured above (figure 2). Root length, whole plant fresh weight, and whole plant dry weight significantly differ ($p < 0.05$) between different treatments and are significantly increased compared to the control (Table 3).

According to the results, the root development of all treatments differs significantly from each other. However, the root length of all treatments except T2 (higher proportion with Pseudomonas fluorescence) differs significantly only from the control. The highest root length was 6.2 ± 0.4 cm in T3, while the lowest at the control (T5) (4.1±1.2cm).

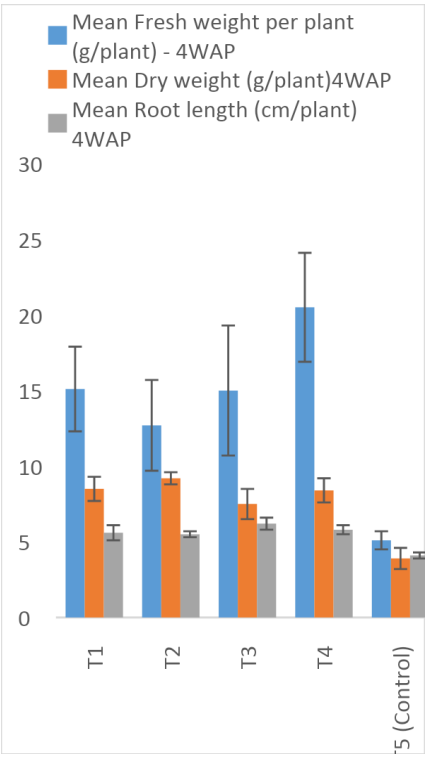

Figure 2. Progress of mean fresh weight, dry weight and root length under different bacteria combinations at 4WAP

Among all the treatments mentioned, T3 was identified as the best treatment for increased root length (6.2 ± 0.4 cm) compared to others. In this treatment, *B. megaterium* was used extensively and the other two bacteria were used in the same ratio. Inoculation with *B. megaterium* affects the root system in plants with the effects of phytohormones including a decrease in primary root growth followed by an increase in lateral root number, lateral root growth, and root hair length in *Arabidopsis thaliana* and *Phaseolus vulgaris* plant [40]. Further bacterial phytohormone secretion can affect root architecture by increasing the number of lateral roots and root hairs, which in turn increases nutrient and water uptake and promotes development.

The effect on cowpea seedlings may be due to the action of bacteria as an inducer of various phytohormones, such as indoleacetic acid (IAA), abscisic acid, organic acid, gibberellins, and cytokinin [41]. These phytohormones promote root growth and increase the number of root hairs [42]. In addition, the *B. megaterium* strain protects plants from diseases [8]. The greenhouse experiments indicated that the method of bacterial application affected the effectiveness with which bacillus strains protected cowpea seedlings from soil-borne pathogens [43]. IAA-producing PGPR strains are often known to increase root length, and increase root surface area, allowing plants to absorb more nutrients from the soil. IAA is responsible for promoting root extension and the division, growth, and differentiation of plant cells and tissues.

As shown in Table 3, fresh weight and dry weight also differ significantly ($P < 0.05$) between all treatments while a fresh weight of T4 (20.5 ± 3.6 cm) differs significantly from control (5.1 ± 0.6 cm). The highest and lowest fresh weights were from T4 and the control respectively. Based on [44], fresh weight determination doesn't assess plant growth promotion by PGPR, it was used to determine dry weight. Thus, the dry weight determination of all treatments is significantly different compared to the control (3.9 ± 0.7), where T4 has the highest dry weight and is abundant in *A. vinelandi*.

The biomass of a plant is accurately measured using dry weight, which eliminates fluctuations caused by water content. Plant performance in response to factors such as photosynthetic capability, nutrition, environmental factors, and more, can be directly correlated to total plant biomass [45]. In addition, phosphate availability in plants is said to be 0.2 % of dry weight [46]. As dry weight increases, phosphate solubilization increases which is essential for photosynthesis, respiration, energy storage, and transmission during cell division and elongation [47]. A key component of phytin, phospholipid, and nucleic acid, phosphate account for about 0.2 percent of the dry weight of plants. In addition, it is essential for photosynthesis, respiration, energy storage, and transfer during cell division and elongation [48].

Overall, all three of these bacteria had a positive effect on plant growth, all parameters of the result of bacterial treatments were always ahead of the

control. This was mainly due to the activation of bacteria. All three bacteria advocated increasing the germination rate of the cowpea plant. This was mainly due to the use of *P. fluorescence* bacteria, which affects the germination rate.

IV. CONCLUSION

PGPR has an immense contribution in plant growth and development as well as in its physiological performances of crops especially fruits and vegetables. External application of PGPR to the seed with the combination of coating solution can provide surplus amount of PGPR to the plant root zone while seed emerges and can be an inducer for the growth and development of the plant with high germination rate. Based on the results obtained from this study, the cowpea seeds coated with PGPR such as P. fluorescence, *A. vinelandi* and *B. megaterium* showed better performance compared to control while investigating all the plant growth parameters tested. Anyhow, based on the ratio of bacterial strains used, the change observed in each plant growth parameter differed significantly among all the treatments. In case of plant height, despite of the fact that the plant height increases with the age of the plant, at 1WAP and 4WAP, *A. vinelandi* and *Pseudomonas fluorescence* significantly exhibited the better growth compared to control. In case of dry weight / biomass, higher proportion of solution with *A. vinelandi* provided the highest dry weight content while root enlargement of plants highly induced by abundant *B. megaterium* in the solution. Therefore, all three bacterial strains coated on cowpea seeds show better plant growth and development compared to the control, and the uniqueness of each bacterial strain decides the level of performance.

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FORMULATION OF FLAKES INCORPORATING SPROUTED GRAIN FLOURS AND RICE BRAN

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Abstract*:* Rice bran (RB) is a by-product of rice milling process. Even though RB is rich in nutrients it is less popular as a food ingredient and commonly used as animal feed in Sri Lanka. Sprouting improves the digestbility and overall nutritionl quality of grains. Thus this study was conducted to develop flake using wheat flour (WF), composite flour (CF) (sprouted chickpea flour, sprouted corn flour and sprouted mung bean flour) and RB. The proportion of sprouted grain flours (3 chickpea flour: 7 corn flour: 2 mung bean flour) was identified as the best CF through the preliminary trials. WF and RB were mixed in five different proportions (100% WF), (83.3% WF, 16.7% RB), (66.7% WF, 33.3% RB), (50% WF, 50% RB) and (100% RB) with a constant amount of the best CF in flake formulation. Flakes with 83.3% WF and 16.7% RB was confirmed as the best WF and RB combination based on the sensory evaluation through one-way ANOVA, Tukey Method. The hardness, fracturability and resilience crunchy in milk values of the finalized flake respectively 7.94 ± 0.06 N, 1.18 ± 0.07 N and 9.05 ± 0.75 minutes and chemical properties were crude fat (12.33 ± 0.38%), crude fiber (2.89 ± 0.00%), crude protein (14.94 ± 0.20%) and carbohydrate (65.56 ± 0.11%). The total phenolic content and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity of the flake was 179.21 ± 7.38 mg (Gallic acid equivalent / 100 g) and IC_{50} (40.65 mg/ml) respectively. Thus, this study enabled to give value addition to RB as an ingredient in food preparation.

Keywords*:* Flakes, Sprouted Grains, Rice Bran, Composite Flour

I. INTRODUCTION

One of the ready-to-eat food items that is frequently utilized as a breakfast menu, is flakes. Usually it is consumed with the addition of milk or water. Wheat, rice, barley, maize, and oats like cereals are frequently used to prepare flakes [1]. However, cereals are often high in lysine and low in important amino acids like threonine and tryptophan. On the other hand, legumes are low in lysine but high in important amino acids, especially those containing sulfur. As a result, adding cereal and legume products together may enhance the nutritional qualities of a meal [2].

Sprouting is an ancient process that increases essential amino acids, protein

digestibility, amino acid availability, certain vitamins such as thiamin, riboflavin, niacin, and ascorbic acid in cereals and legumes [3]. Further, it improves the quality of legumes digestibility and physiological function by breaking down anti-nutrients [4]. Thus, using the sprouted grain flours is a way of enriching nutritional value of flakes.

Rice (*Oryza sativa*) is a staple food for over half of the world's population. Rice bran (RB), as a by-product during rice milling process has carbohydrates, lipids, vitamins like thiamine, riboflavin, and niacin and minerals including phosphorus, potassium, magnesium, calcium, manganese, zinc, sodium and iron [5]. It is also a rich source of proteins, fibers, antioxidants and possesses several health benefits. Since RB proteins are high in lysine, a limiting amino acid in cereal grains, they have a superior amino acid profile, good source of hypoallergenic proteins and highly digestible. Nowadays, dietary fiber-added functional foods are high in demand. Given its reasonable dietary fiber content, which ranges from 20-30%, RB can be used as functional food ingredient [6]. Thus the present study was aimed to formulate flakes incorporating sprouted grain flours and RB.

II. MATERIALS AND METHODS

Materials: wheat flour (WF), chickpea, corn kernels, mung bean, carrot, margarine, baking powder, sugar and salt were purchased from local markets. RB was purchased from local rice mill, sieved and stored in refrigerator.

A. *Preparation of sprouted grain flours*

Chickpea, corn kernels and mung beans were sorted to remove foreign material, damaged and aborted seeds and washed under running tap water separately. Washed grains were soaked overnight (24 hours) in water in glass container separately. Sprouting grains were sprinkled with water to maintain adequate hydration for 48 hours. Sprouted grains were dried in an oven at 60 °C for 12–20 hours separately. Dried sprouts were milled to flour and sieved [7].

B. *Preparation of dehydrated carrot powder*

Carrots were washed, peeled and cut into small pieces. The carrot slices were blanched in hot water at 95 °C for 5 minutes, slices taken out immediately dipped in cold water and allowed to drain. Slices dried in an oven at 50 °C for 10 hours and powdered [8].

C. *Identification of the best composite flour (CF) combination*

Four CF samples were prepared according to the following mixing ratio: 10:7:3:2 (sample code 726), 10:3:7:2 (sample code 358), 10:2:3:7 (sample code 921) and 10:4:4:4 (sample code 543) of WF, sprouted chickpea flour, sprouted corn flour and sprouted mung bean flour respectively. Then each formulation mixed with 3 g of dehydrated carrot powder, 10 g of sugar, 1 g of salt, 10 g of margarine and 0.1 g of baking powder to make the flake dough. Dough was flattened, molded and the flakes were baked using an oven at 120 °C for 20 minutes. Sensory evaluation

was conducted to identify the best combination of CF.

D. Formulation of flakes

Five formulations {100% (30 g): 0% (0 g) (sample code 352), 83.3% (25 g): 16.7% (5 g) (sample code 765), 66.7% (20 g): 33.3% (10 g) (sample code 564), 50% (15 g) : 50% (15 g) (sample code 291) and 0 % (0 g) : 100% (30 g) (sample code 643)} were prepared by mixing WF and RB respectively. Each formulation was mixed with 36 g of best CF, 3 g of dehydrated carrot powder, 10 g of sugar, 1 g of salt, 10 g of margarine and 0.1 g of baking powder to prepare the flake dough. Dough was then flattened, molded and the flakes were baked in an oven at 120 ^{0}C for 20 minutes.

E. Sensory evaluation of flakes

Five formulations of flakes formulated using WF, CF and RB were tested for sensory attributes using nine-point hedonic test with 30 panelists in Food Science and Technology Department of Sabaragamuwa University of Sri Lanka.

F. Proximate analysis

Proximate analysis of the flakes performed using Association of Official Analytical Chemists, 2000 methods [9].

G. Evaluation of antioxidant capacity of flakes

1) Preparation of methanol extracts of flakes: Methanol extracts of flake was prepared based on the procedure described in Jan *et al.*, [10] with some modifications. Three grams of powdered sample was taken into beaker and added 100 ml of methanol. The mixture mixed for 2 hours using magnetic stirrer, centrifuged for 10 minutes at 3500 rpm and methanol was evaporated at 40 ^{0}C. Thus obtained extract was stored in refrigerator.

2) DPPH radical scavenging activity: Scavenging activity was determined Based on the procedures described by Kumara, K and Kumar B, [11] with some modifications. 0.5 ml of 0.2 mg/ml solution of DPPH was added to test tubes containing 0.5ml, 1.5 ml, 2.5ml, 3.5 ml and 4.5 ml of extract respectively. Final volume of test tubes were made up to 5 ml using methanol. Test tubes were shaken vigorously and allowed to stand at room temperature in dark. Absorbance was measured immediately at 520 nm by UV spectrophotometer and experiment was done in triplicate. Methanol was used as the blank. Same procedure was done to ascorbic acid to plot the ascorbic acid standard curve by adding 0.5 ml of 0.2 mg/ml solution of DPPH to 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml, 1.4 ml, 1.6 ml, 1.8 ml, 2.0 ml of 0.1 mg/ml solution of ascorbic acid. Final volume was made up to 5 ml using methanol. Percentage of DPPH scavenging effect was calculated using the following equation.

$$\% \text{ Inhibition} : A_0 - A_1 / A_0 \ 100$$

where, A_0 = The absorbance of control.

A_1 = The absorbance of sample/ standard

H. Total phenolic compounds assay

The total phenolic compounds of extracts were determined with the folin-ciocalteu reagent based on the procedure described in Jawad Kadhim *et al.*, [12]. The total phenolic compounds were calculated and expressed as mg Gallic acid equivalent (mg GAE)/100g).

I. Analysis of physical characteristics

1) Colour Analysis: The colour of the flake was determined using a chroma meter based on the procedure described in Kince *et al.*, [13] in L*, a*, b* colour scale. Measurement of L*=lightness (where 0 = black, 100 = white), a* (+a* = redness and -a* = greenness) and b (+b* = yellowness and -b* = blueness). Ground flakes were used for the determination of colour.

2) Texture analysis: The texture was measured based on procedure described in Wanyo, Chomnawang and Siriamornpun [14]. Texture profile analysis of flake was performed at room temperature by using a Brookfield CT3 texture analyzer with trigger Load: 15.0 g and test Speed: 1.00 mm/s.

3.Resilience crunchy in milk: Resilience crunchy in milk was measured based on the procedure described in Anandito et al [1]. The flake was kept in milk and calculated the time until the crispness disappears.

J. Shelf-life evaluation

For storability test, flakes were packed under hygienic condition in clean packages and sealed immediately to protect product quality. Polythene bags (LDPE), aluminum foil and glass were used for packaging. During shelf life study, sample was kept at room temperature (27 °C and RH 60%) until all analysis were performed. The shelf life of flake was evaluated based on the microbial count.

K. Statistical analyses

One-way ANOVA, Tukey method was used to analyze the sensory score using Minitab 19 statistical software at a 5 % level of significance. Data was presented as mean ± SD.

III. RESULTS AND DISCUSSION

A. Sensory analysis

Table 01 shows the sensory score for appearance, aroma, crispiness, crunchiness, taste, after taste and overall acceptability of CF flakes. There was no statistically significant difference for appearance, aroma, colour, crispiness and crunchiness observed among the coded samples 726, 358, 921, and 543. However, coded sample 358 showed high sensory score in taste, after taste and overall acceptability than other coded samples. Thus, the CF proportion in sample coded 358 (3 sprouted chickpea flour: 7 sprouted corn flour: 2 sprouted mung bean flour) were finalized as best CF and used in the formulation of flakes.

Table 1. Identification of best composite flour combination through sensory score of composite flour flakes

Sensory Attributes	*p* value	Sensory Score			
		726	358	921	543
Appearance	0.000	7.80±0.93[a]	8.27±0.64[a]	6.97±0.10[b]	6.97±1.03[b]
Aroma	0.000	7.83±0.97[a]	7.87±0.78[a]	6.83±1.05[b]	6.90±1.13[b]
Colour	0.000	7.90±1.06[a]	8.40±0.56[a]	6.97±0.89[b]	7.10±1.09[b]
Crispiness	0.000	7.73±1.14[a]	8.27±0.74[a]	7.00±1.17[b]	6.93±1.20[b]
Crunchiness	0.000	7.87±0.97[a]	8.40±0.62[a]	7.17±1.21[b]	7.07±1.21[b]
Taste	0.000	7.73±0.83[ab]	8.23±0.77[a]	7.07±1.31[bc]	6.77±1.14[c]
After taste	0.000	7.50±1.08[ab]	7.97±0.99[a]	6.87±1.22[bc]	6.70±1.02[c]
Overall acceptability	0.000	7.63±0.85[b]	8.43±0.57[a]	7.03±0.81[c]	6.60±0.93[c]

Values followed by the same letters in the same row are not significantly different ($p \leq 0.05$)

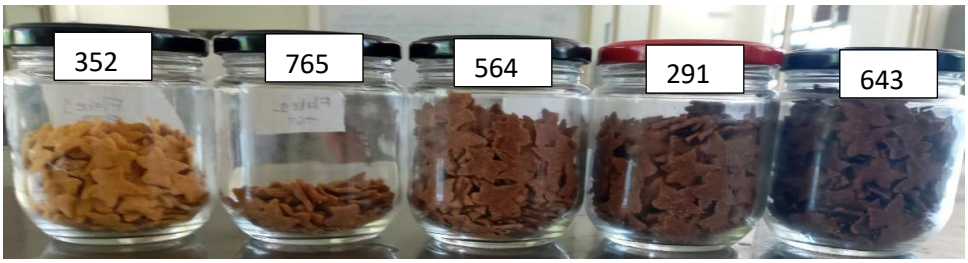

Figure 1. Five formulations of flakes

Table 02 shows the sensory score of flakes for appearance, aroma, colour, consistency, mouth feel, taste, after taste and overall acceptability. Even though there was no statistically significant difference in appearance, colour, consistency between coded samples there was a high sensory score for coded sample 765 (WF: RB, 83.3 %: 16.7 %) than all other coded samples. Therefore, proportion of ingredients present in coded sample 765 was selected as ingredients for final product.

Table 2. Sensory score of formulated flakes

Sensory Attributes	P value	Sensory Score				
		352	765	564	291	643
Appearance	0.000	7.83±1.05^{a}	8.03±0.89^{a}	7.03±0.81^{b}	6.30±0.92^{c}	5.63±1.16^{c}
Aroma	0.000	7.43±0.86^{ab}	8.03±0.93^{a}	6.73±0.91^{bc}	6.23±0.89^{cd}	5.9±1.27^{d}
Colour	0.000	7.47±1.31^{a}	8.01±0.83^{a}	6.67±0.55^{b}	6.30±0.70^{b}	5.53±1.33^{c}
Consistency	0.000	7.50±0.73^{a}	7.90±0.92^{a}	7.27±0.74^{a}	6.37±1.10^{b}	6.07±1.31^{b}
Mouth feel	0.000	7.37±0.81^{ab}	7.87±0.94^{a}	6.87±0.97^{b}	6.07±1.11^{c}	5.60±1.25^{c}
Taste	0.000	7.50±0.82^{ab}	8.17±0.91^{a}	6.87±0.97^{b}	6.03±0.96^{c}	5.50±1.14^{c}
After taste	0.000	7.47±0.78^{ab}	8.10±0.85^{a}	6.87±0.90^{b}	5.97±0.76^{c}	5.50±1.14^{c}
Overall acceptability	0.000	7.78±0.63^{b}	8.37±0.77^{a}	6.90±o.71^{c}	6.20±0.71^{d}	5.60±0.97^{e}

Values followed by the same letters in the same row are not significantly different ($p \leq 0.05$)

B. Proximate composition

Table 3. Proximate composition of flakes

Moisture %	Ash%	Crude Fat%	Crude Protein%	Crude fiber%	Carbohydrate%
2.20±0.06	2.08±0.05	12.33±0.38	14.94±0.20	2.89±0.00	65.56±0.11

Values are expressed as means ± standard deviation ($n = 3$)

The formulated flake was found to be of good quality (Table 03) according to the quality parameters (maximum moisture content about 3.0%, maximum ash content of 4.0%, minimum fat content of 7%, minimum protein content of 5.0 %, minimum carbohydrate content is 60%) mentioned in Anandito RBK et al's research paper [1].

It was observed that carbohydrate was predominant and protein and mineral content was high in the formulated flake. This must be due to the addition of sprouted chick pea flour, sprouted corn flour [15], sprouted mung bean flour [16] and RB [5]. The increase in protein value indicates high nutritional value. Further the good fiber percentage could have come from RB and sprouted grains [17].

C. Physical characteristics of flake

Table 04 shows the physical characteristics of the developed flake. Hardness of a flake is the force required to break the flake and it was found to be 7.94N. This result was lower than the force that required to break the flake that was formulated using WF, rice flour and RB [14]. This was due to the use of sprouted grain flours as an ingredient in this research study [18]. Fracturability refers to the tendency of breakability and it was identified as 1.18 N which was lower than the cereal flakes prepared by Anandito RBK et al. [1].

The length of time that a product's texture remains consumer-acceptable after being soaked in milk is one of the most crucial quality criteria. Crisp resistance in milk was the time for flakes to be able to floating on the surface of milk until the texture was not crispy. The crisp resistance in milk of flake should be more than three minutes [1] and this product showed good resilience crunchy in milk (nine minutes).

Figure 01 shows the colour variation of the formulated flakes and the effect of rice bran proportion in the colour. The colour attribute of this study almost same as the study conducted by Wanyo et al. [14] which stated that L* = 57.02, a*=8.81 and b* 33.18 for cereal flake composed of (WF: Rice flour : RB, 40 : 0: 60). This may be due to the incorporation of common ingredient (RB) in both research [14]. The presence of anthocyanin pigment in bran layer provides the characteristic dark purple [19].

D. Shelf life analysis

Table 05 shows the shelf life analysis of formulated flakes. Total plate and yeast and mold count provides specific details about the food's microbial quality. Microbiological limits for dried and instant processed cereal products requiring reconstitution; Aerobic plate count per gram: 5 x 10^4, Yeasts and moulds count per gram: 1 X 10^2 (Food Act No. 26 of 1980) [20]. Based on the results, formulated flake was within different packaging material showed good microbial stability and It was concluded that the aluminum packaging was most suitable than glass and polythene packaging.

Table 4. Physical characteristic of the flakes

Hardness (N)	Fracturability (N)	Resilience Crunchy in Milk (minutes)	Colour attribute		
			L*	a*	b*
7.94 ± 0.06	1.18 ± 0.07	9.05±0.75	57.79±0.68	8.67±0.89	33.11±6.91

Values are expressed as means ± standard deviation (*n* = 3)

Table 5. Shelf life analysis

Attribute	0 days	After two week		
		Aluminium	Glass	Polythene
Total plate count (CFU/g)	N/A	1.67 ×10^2	2.66 ×10^2	3.67 ×10^2
Yeast and mould count (CFU/g)	N/A	0.3×10^2	0.6 ×10^2	0.7 ×10^2

E. Antioxidant capacity

Table 6. Antioxidant capacity

(mg GAE/ 100g)	DPPH-IC_{50} (mg/ml)
179.21±7.38	40.65±0.06

Values are expressed as means ± standard deviation (*n* = 3)

Table 06 shows the antioxidant capacity of the developed flake. The phenolic compounds' abilities to function as reducing agents, free radical scavengers, and hydrogen donors are largely responsible for the antioxidant potentials in plant foods. Dietary phenolic antioxidants have the potential to significantly slow the progression of many chronic diseases linked to oxidative stress, including cancer, inflammatory bowel syndrome, neurological diseases, and the aging process [21].

The formulated flakes showed the total phenolic content as 179.21 ±7.38 mg (GAE/ 100g) and the IC_{50} value was 40.65 ±0.06 mg/ml in DPPH free radical scavenging assay. The IC_{50} of ascorbic acid was 0.027 ±0.00 mg/ml. RB consisted significant amount of Oryzanols, tocopherols, tocotrienols, phytosterols and phenolic compounds which exhibit antioxitant activity. Cereals naturally contain polyphenols which exhibited strong antioxidant property. Further, Germination process increases the phenolic content of legumes [22]. The increase in antioxidant activity may also be due to the presence of carrot which is high in beta-carotene, an antioxidant that helps the body fight infections, keep eyes healthy, and replenish epithelial tissues in the lungs and skin [23].

IV. CONCLUSION AND RECOMMENDATION

RB a by-product of rice milling process is commonly used as animal feed in Sri Lanka can be used as raw material in flake making. This study has demonstrated that addition of RB along with the sprouted grains (chickpea, corn, mung bean) increases fiber contents in formulated flakes and increase of fiber contents may contribute to health benefits, for example increasing faecal bulk and lowering of plasma cholesterol. With the nature of the flake formulation composition flakes were found to be good source of protein, predominant in carbohydrate and exhibited high antioxidant activity. Further, the flakes showed good crisp resistance value in milk and microbial stability in aluminium packages. More studies are warranted to identify the shelf life evaluation, keeping quality parameters with storage and phytochemical composition of the flakes.

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ESTIMATION OF QUALITY PARAMETRS OF COMPOST AVAILABLE IN THE MARKET AND QUANTIFICATION OF CROP REQUIREMENTS OF COMPOST BASED ON GUIDELINES OF DEPARTMENT OF AGRICULTURE, SRI LANKA

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Abstract: The production of compost provides a solution for recycling organic waste and reduces the use of chemical fertilizer for the crop cultivation. In Sri Lanka, recommendations for the quantity of compost to be used for different crops based on nutrient composition are not available. Hence, commercially available compost samples of 1 kg (n=30) were collected from randomly selected sellers in Sri Lanka to quantify total nitrogen, phosphorous, and potassium contents and other quality parameters as per SLS 1634: 2019 and SLS 1635:2019 standards. Furthermore, the quantity of organic fertilizer to supply nitrogen, phosphorous, and potassium requirements for different crops was calculated based on recommendations provided by the Department of Agriculture of Sri Lanka for inorganic fertilizers. Among the tested samples moisture(43%), pH(40%), EC (47%), sand(87%), total nitrogen(37%), total phosphorous as P_2O_5(40%), total potassium as K_2O(50%), organic carbon as C(83%), total Mg as MgO(53%), total Ca as CaO(37%) do not comply with requirements specified in SLS 1634: 2019 and SLS 1635: 2019 whereas potential toxic elements (Cd,Pb,Cr,Ni,Hg,As) contents of all the tested samples comply. The rrequired amount of compost is considerably higher than that of chemical fertilizer as per calculations based on fertilizer recommendations by the Department of Agriculture. Therefore, it is essential to promote and facilitate large-scale compost production with a proper regulatory mechanism to improve quality. Nutrient composition and quality parameters of tested samples vary in a wide range. It is necessary to establish regulations and standards for compost production targeting different purposes such as organic fertilizer, soil fertility enhancers, landfill materials, fertilizer for reclamation sites, soil amendments, or mulches. Since compost alone cannot fulfil the fertilizer requirement of crops to feed a whole nation, it is recommended to sell the harvest separately at an elevated price to cater to people who require organically produced food.

Keywords: **Nitrogen, Organic fertilizer Phosphorous, Potassium, Quality**

I. INTRODUCTION

Increase in waste generation and lack of infrastructure for waste management has become a huge issue during recent decades in Sri Lanka. Currently in Sri Lanka, collection of municipal waste only covers the urbanized and commercial areas. Much of the collected solid waste is openly dumped into waterways, vacant fields; landfill sites which are usually located close to streams, marshes or forested areas and can harm the environment and public health [1,2,3]. Composting helps to reuse organic waste to return nutrients back to the soil which leads to reduce the cost of fertilizers [1]. For an example, in 2020 government of Sri Lanka imported and distributed 550,846,030 kg of inorganic fertilizers free of charge among the farmers [4]. It is estimated that recycling organic waste leads to reduce the cost of waste management and inorganic fertilizers by United States dollar 191 million to 357 million [5]. Production of compost was encouraged by the government of Sri Lanka with the objectives of providing a solution for recycling organic and municipal solid wastes, providing the organic fertilizers to farmers, and reducing synthetic fertilizer requirements for cropping.

Compost production in Sri Lanka has changed from small-scale at farms or home gardens to large-scale composting at the commercial level whereas the raw material used for compost preparation has changed and diversified from household organic waste to municipal solid waste during previous years. Production and usage of organic fertilizers, remain less popular among farmers compared to inorganic fertilizer. Seasonal variation in the availability of raw materials such as crop residues, increased requirement of labour, and prevalence of chemical fertilizers on the market seems to be the major barriers that limit the production of compost within the Sri Lankan context. Unlike compost, crops response quickly to inorganic fertilizer since they are dense in nutrients. To obtain the desirable effects, comparatively to inorganic fertilizer, compost is required to apply in large quantities. Most of people are not aware of the benefits of compost for soil fertility. As a result of these combined effects, farmers and cultivators have negative impacts and poor interests in the usage of compost. However, compost is extensively used by the people engage in the floriculture industry, nursery management, and home gardening, although cultivators do not use compost widely in the commercial-scale cultivations of vegetables and other annual crops [6]. However, Composting combines environmental protection with sustainable agricultural production associated with climate-smart agriculture. Compost plays a significant role in organic farming. In other countries and even in Sri Lanka, organic foods are sold separately for an elevated price.

The 8-year economic development plan 'Vision 2025 – A Country Enriched' which was implemented by the government of Sri Lanka in the year 2017, stated to develop a national policy on the use of permitted levels of fertilizers, increase the share of organic products in the market, and improve solid waste management [7,8]. Therefore, the

government banned the importation of synthetic fertilizers, and agrochemicals on or after May 6, 2021. Although Sri Lankan government aims to produce organic fertilizers to cater the demand for inorganic fertilizer requirements, the government did not intervene to increase organic fertilizer production capabilities [9]. In addition to that, there is not any proper mechanism to regulate the price and quality of compost in Sri Lanka although there are standards for the specifications for compost.

Compost is a stable decomposed or processed product which shows similar characteristics as humus. Humus is generated due to the decomposition of biodegradable substances. Composts consist of plant nutrients. It may contain mineral materials [10,11]. Compost can be prepared by using agricultural wastes and or by using municipal solid wastes. Other than using as a fertilizer to supply nutrients, compost is used as a soil conditioner or landfill material in reclamation sites [10, 12,13,14].

In Sri Lanka, limited numbers of studies have been done to evaluate and quantify the nutrient composition of compost those commercially available in the market irrespective of the source of origin. None of the recommendations were made and cultivators were not aware of the quantity of compost to be added to crops in Sri Lanka based on the actual nutrient composition of composts and actual nutrient requirements of crops. It created a catastrophic situation that significantly minimizes the yield of harvest. Therefore, the objectives of our study were to evaluate and quantify nutrients and potentially toxic elements in organic fertilizer available in the market irrespective of the source of origin, to compare the total nitrogen, phosphorous, and potassium contents of composts with those of inorganic fertilizer followed by making recommendations on required quantity of organic fertilizer to apply for the crops based on the recommendations provided by the Department of Agriculture.

II. METHODOLOGY AND EXPERIMENTAL DESIGN

Sample Collection

Urea (n=6), Murate of potash (n=6), Tripple super phosphate (n=6) were randomly collected from the chemical fertilizer available in the market. Compost samples of 1 kg (n=30) were collected from the market, from randomly selected compost producers in Sri Lanka irrespective of the sources of composted materials. Collected samples were labeled and stored at ambient temperature, until analyses are carried out.

Sample preparation and analysis

All organic and inorganic fertilizer samples were grounded separately using a domestic mixer grinder (Philips HL7699/00, India) and homogenized thoroughly by cone and quartering method prior to analysis. Analytical grade chemicals, deionized water, and A-grade glassware were used during the analysis of fertilizer samples. All the analyses were done in duplicates. During the analysis, reagent blanks, in house prepared control samples with known assigned values were analyzed for each parameter to assure the accuracy of test results. During the analysis of results,

central tendency, data distribution, and deviations of each parameter were identified after eliminating outliers.

Moisture Content

Moisture content of fertilizer samples were quantified as to SLS 645:Part 02:1984 [15] by taking the weight difference of approximately 10 g of prepared sample before and after drying at 105±2 °C for 5 hours at a calibrated hot air oven (UF160, memmert, Germany).

Total Nitrogen Content

Total nitrogen (TN) content was determined by Kjeldhal method as to SLS 645:Part 01:2009 in urea samples [16]. To quantify nitrogen content in urea samples, they were digested in the presence of Kjeldhal catalyst (VELP Scientifica, Italy) and concentrated sulphuric acid using heating digester (DKL 12 Automatic Digestion Unit, VELP Scientifica, Italy) distilled to sulphuric acid solution (25 mL, 0.5 mol L^{-1}) using automatic distillation unit (UDK 149 Automatic Kjeldahl Nitrogen Protein Analyzer, VELP Scientifica, Italy) and titrimetrically quantified using standard NaOH (0.5 mol L^{-1}) solution in the presence of methyl red indicator.

To determine total nitrogen content of organic fertilizer samples, nitrate (NO_3^{2-}) content was determined using Devarda method (AOAC 892.01, 1932) [17]. For that sample (0.5g) and Devarda Alloy (5 g) was distilled using automatic distillation unit (UDK 149 Automatic Kjeldahl Nitrogen Protein Analyzer, VELP Scientifica, Italy) and titrimetrically quantified using standard NaOH solution (0.5 mol L^{-1}) in the presence of methyl red indicator.

Kjeldhalnitrogen content of organic fertilizer was quantified by Kjeldhal method. For that, accurately weighed sample (1 g) was digested in the presence of Kjeldhal catalyst (VELP Scientifica, Italy) and concentrated sulphuric acid using heating digester (DKL 12 Automatic Digestion Unit, VELP Scientifica, Italy), distilled to sulphuric acid (25 mL,0.5 mol L^{-1}) using automatic distillation unit (UDK 149 Automatic Kjeldahl Nitrogen Protein Analyzer, VELP Scientifica, Italy) and titrimetrically quantified by using standard NaOH solution (0.5 mol L^{-1}) in the presence of methyl red indicator. Total nitrogen content is the sum of nitrate (NO_3^{2-}) nitrogen and Kjeldhal nitrogen content.

Total Phosphorous Content

Total phosphorous (P) content of tripple super phosphate (TSP)and organic fertilizer samples was quantified using a UV Visible spectrophotometer (Cary 100, Agilent Technologies, United State of America) by molybdovanadophosphorous method as to SLS 645: Part 5:1985 [18] after acid digestion of samples. Standard phosphate solution series with known phosphate concentrations were used to calibrate the UV Visible spectrophotometer. Prepared samples (5 g) of TSP fertilizer, were boiled in the presence of concentrated hydrochloric acid (10 mL) and filtered through slow filter paper. After adding calcium oxalate (1 g) to accurately weighed organic fertilizer (5 g) samples, the mixture was calcined at 450 °C using a calibrated muffle furnace (L24/12/B180,

Nabertherm, Germany) and cooled to room temperature before boiling in the presence of concentrated hydrochloric acid (10 mL) and filtered through slow filter paper. To a known volume of this solution, a known volume of vanadomolybdate reagent was added, after diluting to the mark of the volumetric flask, absorbance was measured at 420 nm after allowing to stand the solutions for 10 minutes at 27±2 °C.

Total Potassium Content

Total potassium (K) content of murate of potash (MOP) quantified as to SLS 645: Part 5;1985 [19] using a flame photometer(model 420, Sherwood Scientific Limited, United Kingdom)after acid digestion of samples. For that, the concentrated hydrochloric acid (10 mL) and distilled water (50 mL) were added into MOP samples (2.5 g) and evaporated to dryness. To each of these solutions, saturated ammonium oxalate (50 ml) was added and boiled for 30 minutes. After adding slight excess ammonia solution, solutions were filtered with a slow filter paper, diluted to volume, and aspirated using the flame photometer. To quantify potassium content in organic fertilizer samples, homogenized and prepared samples (10 g) were incinerated using a calibrated muffle furnace (L24/12/B180, Nabertherm, Germany) at 450 °C for 16 hours and cooled to room temperature. The solutions were prepared, and potassium content was quantified by following the same procedure as for MOP.

pH

In the organic fertilizer samples, pH was measured at 25±2 °C according to the method ISO 10390: 2005 [20] by shaking the sample for 60±10 minutes after dissolving the sample in distilled water to 1:5 (Volume:Volume) using the calibrated pH meter (WTW pH 7110,inoLab,Germany).

Electrical Conductivity

According to ISO 11265:1994 [21], the specific electrical conductivity of organic fertilizer samples was measured at 27±2 °C in the filtrate obtained after shaking the sample for 30 minutes after dissolving the sample in distilled water to 1:5 (Mass:Volume)ratio using the calibrated conductivity meter (Cond 7110, inoLab, Germany).

Sand Content

Sand content of each organic fertilizer sample was measured gravimetrically, after a thorough washing of the sample (100g) using distilled water, followed by drying at 103±2°C for one hour using calibrated oven [10,11] (UF160, memmert, Germany).

Magnesium and Calcium content

Magnesium and calcium content of the organic fertilizer samples were quantified by titrating the sample with EDTA as to SLS 645: Part 6: 1990 [22]. The prepared sample (5 g) was incinerated at 450°C using a calibrated muffle furnace (L24/12/B180, Nabertherm, Germany) for 16 hours following acidification with concentrated hydrochloric acid, boiling and filtered and made to the mark (1000 mL). Then the prepared solution (10 mL)

was pipetted to a titration flask, added distilled water (100 mL), and neutralized with the ammonia
solution (10%, volume: volume). After adding potassium hydroxide – potassium cyanide solution (10 mL) and HHSNNA indicator (Patton and Reeder's Indicator) (25 mg) of, the solution was titrated with standard EDTA (ethylene diammine tetra acetic acid) solution to determine calcium content. To determine magnesium content, pH 10 buffer (5 mL) and potassium cyanide solution (2 mL), and Eriochrome black T indicator (25 mg) was added to the titration flask which contains prepared solution (10 mL) and distilled water (100 mL). The solution was titrated with standard EDTA solution to determine magnesium content.

Organic Carbon Content

Organic carbon content of organic fertilizer samples was quantified using Walkley –Black method as per the SLS 1634: 2019 and SLS 1635:2019 [10,11]. The sample (0.025g), was transferred to a flask that contain potassium dichromate solution (10mL, 0.1667 molL^{-1}) and concentrated sulfuric acid (20mL). After adding phosphoric acid (10mL 85%) and diphenylamine indicator (1mL), samples were titrated with a ferrous sulphate solution (0.5 mol L^{-1}).

Potentially Toxic Elements

Potentially toxic elements (cadmium, chromium, lead, mercury, nickel, and arsenic) of organic fertilizer samples were quantified using microwave-assisted acid digestion by microwave digester and detection by the Inductively Coupled Plasma Mass Spectrometer. For that, accurately weighed sample (0.25 g) and trace element grade concentrated nitric acid (10 mL) were digested using a microwave digester (MAS 5, CEM, USA). After filtering through Watman 542 filter paper to 25 mL volumetric flask the volume was made up to mark with deionized water. and arsenic content of the collected compost samples were quantified by the Inductively Coupled Plasma Mass Spectrometer (7900 Agilent Technologies, Japan) after calibrating the instrument with a multi element calibration standard series. To assure the quality of test results, reagent blank and spiked samples at a concentration of 50 % of the highest calibration standard of each metal were analysed with each batch of sample.

III. RESULTS AND DISCUSSION

Commercially available compost samples were collected from the market irrespective of the origin for the current study to evaluate the quality of compost. From the compost samples analysed, the percentage that comply and do not comply with the requirements for moisture content, pH at 25±2°C, electrical conductivity at 27±2°C and sand content with respective to Sri Lanka standard 1634: 2019 and Sri Lanka standard 1635: 2019 were depicted in Table 01.

Table 1. Moisture, pH, Electrical Conductivity, and sand content of compost samples

Characteristics	Moisture g/100 g	pH 1:5,V/V*	EC/25.0°C 1:5,W/V** dS/m	Sand content, (on dry basis) g/100 g
Average± standard deviation (n=30)	26.4 ± 11.9	8.3± 0.6	5.3±5.6	39.2± 17.3
Range	3.2- 47.5	6.6-8.9	0.6-17.6	12.9-76.8
Requirement as per SLS1634: 2019 and SLS1635: 2019	Maximum 25	6.5 – 8.5	Maximum 4.0	Maximum 20
% Do not comply with requirement in the standard	43	40	47	87
% Comply with requirement in the standard	57	60	53	13
Note V/V*-volume/volume W/V**-weight/volume				

According to table 01, 43% of tested compost samples contain more than the specified moisture content in both SLS 1634: 2019 and SLS 1635: 2019. The moisture content of compost affects its bulk density. Compost with higher moisture content generates practical issues in transportation, handling, and application in the field. Normally compost with low moisture content is dusty while compost with high moisture content is heavy and clumpy [23]. Moreover, when the moisture content is significantly high, nutrient availability per kilogram of compost becomes low. Therefore, higher quantities of compost have to be applied to fulfil the nutrient requirement of crops. Applying compost with higher moisture content will reduce the yield per hectare and the quality of crops which will cause economic losses. Since farmers purchase compost by paying for the weight of compost, purchasing compost with higher moisture content significantly affect on the profitability of farmers directly and indirectly. If the compost looks too soggy, it is recommended to add dry materials during composting to reduce the moisture content [24].

The pH values of the analyzed samples vary from 6.6 to 8.9. However, 40% of the samples do not comply with the requirements in SLS 1634: 2019 and SLS 1635: 2019 (pH 6.5-8.5). The pH of compost indicates acidity or alkalinity. Certain plant species require a specific pH range and the amount of compost applied and its pH, can affect the soil or growing medium pH [23]. When organic fertilizer is completely cured, it may contain elevated concentrations of organic acids. Presence of excessive amounts of organic acids reduces the pH of compost causing them to be acidic. At the early stages of composting, organic acids are produced, but they should no longer be present at later stages of

composting. Since organic acids can be phytotoxic, low pH values, are undesirable in compost [23]. Adding lime or wood ash, and remixing compost helps to increase pH in such circumstances [24]. The pH of compost affects nutrient availability. For example, ammonia (NH_3) is capable of diffusing through plant membranes and interfering with plant metabolism. The pH determines the balance between NH_3 and NH_4^+ [25]. Therefore, it is essential to regulate pH of compost prior to apply in the field.

The electrical conductivity of analysed samples varied from 0.6-17.6dS/m. The average electrical conductivity (5.3±5.6dS/m) of the tested samples exceeded the maximum allowable limit (4 dS/m) of SLS1634: 2019 and SLS 1635: 2019. Compost samples that have elevated electrical conductivity values can be especially rich in nutrients, since nutrients such as nitrate, ammonium, phosphate, and potassium, are responsible for much of the measured conductivity [26]. Electrical conductivity is an accurate, indirect method of measuring salinity and implicate with the salinity of compost [27]. Salinity which is caused by dissolved ions creates an osmotic gradient that prevents the uptake of plant nutrients and water. If the plant intakes particular ions in an excessive amount due to increased salinity, the excess can be toxic on its own or by displacing more vital nutrients in the plant [28]. However, most of the soluble salts are soluble nutrients, so compost with a high salt concentration can be considered a good source of nutrients when applied at a low rate.

If composts consist of a considerably higher number of inert materials, such as soil or sand, then these composts will not provide advantages as composts that are dense in organic materials. According to the findings, nearly 90% of the tested samples contain sand more than the maximum allowable limit (20%) of SLS 1634: 2019 and SLS 1635: 2019. The presence of an excessive amount of sand in organic fertilizer reduces their quality. Sand can be an intentional or unintentional adulterant for compost. When compost is prepared on bare ground, soil or sand is mixed into compost during turning, which reduces the organic matter content in the compost. Intentionally sand is added to increase the weight of compost to gain much profitability which in turn reduces the nutritional quality. Therefore, it is important to control the quality of compost via a regulatory body and develop a mechanism for pricing compost based on nutritional quality. Since the nutritional quality of compost can vary from batch to batch, it is essential to analyse the nutritional quality of each batch prior to pricing.

Levels of potentially toxic elements available within tested compost samples are given in table 02. Although organic fertilizers are considered as one of the beneficial sources of nutrients and organic matter, it is believed that organic fertilizers can also be a potential source of environmental pollution due to the presence of potentially toxic elements including lead, arsenic, chromium, cadmium, mercury, and nickel. If organic fertilizers are applied to an agricultural area, some trace metals accumulate in agricultural lands, some of which could

be transferred to the human body [29,30]. According to the findings of China, it was found that the trace metal contents of organic fertilizers exceeded the maximum allowable limits [31,32,33,34]. However, all the tested compost samples comply with the limits given for potentially toxic elements in SLS 1634: 2019 and SLS 1635: 2019. The harmful substances and pollutants such as toxic metals should be below the critical level (CL) in composts [35]. Hence, it is essential to control presence of harmful substances in composts for safe use and not to endanger soil quality, plant growth, food quality, or human health.

Table 2. Levels of potentially toxic elements of compost samples.

Element (mg/kg dry mass)	**Lead**	**Arsenic**	**Chromium**	**Cadmium**	**Mercury**	**Nickel**
Average	2.3 ±1.4	0.8±0.6	7.6 ±5.0	0.1±0.1	ND*	4.0 ±3.2
Range	0.0-6.8	0.0-2.9	0.0-19.3	0.0-0.5	ND*	0.0-12.3
Requirement (Maximum) as per SLS1634: 2019	50	3	50	3	0.5	50
Requirement (Maximum) as per SLS 1635: 2019	150	5	150	3	2	50
% Comply with both standards	100	100	100	100	100	100
Note- ND*-Not Detected Limit of detection of mercury is 0.1 mg/kg						

Nitrogen, phosphorous, and potassium are considered the primary nutrients which are essential for the growth and development of crops. Total nitrogen content of urea, total phosphorus content in TSP, and total potassium content in MOP were given in table 03. Unlike these chemical fertilizers compost consists of a variety of plant nutrients including nitrogen(N), phosphorus(P), potassium(K), calcium(Ca), magnesium(Mg), and organic carbon(C). These nutrients are also essential for the growth and development of crops. Table 04 contains the average nutrient content and the percentages of tested compost samples that comply with each standard.

Table 3. Average nitrogen content in urea, phosphorous content in TSP, and potassium content in MOP.

Parameter	**Value (g/100g)**	**Requirement for fertilizer grade**
Average Total Nitrogen content as N, on dry basis in Urea	46.1 ± 0.1	Minimum 46 g/100g as to SLS 618:2014 Specification for urea
Average Total Phosphates as P_2O_5 – in TSP	47.6 ± 0.7	Minimum 46 g/100g as to SLS 812:2014 Specification for triple super phosphate
Average water-soluble potassium content as K_2O, in MOP	61.1 ± 0.4	Minimum 60 g/100g as to SLS 644:2014 Specification for potassium chloride

Table 4. Total nitrogen, phosphate, potassium, calcium, magnesium, and organic carbon content of tested compost samples on dry basis (d/b).

Unit- **g/100g**	**Total N**	**Total P as P_2O_5**	**Total K as K_2O**	**Organic Carbon as C**	**Total Mg as MgO**	**Total Ca as CaO**	**C:N ratio**
Mean ± STD	1.8±1.4	1.7±1.4	1.5 ± 1.2	13.2 ± 6.7	0.7±1.3	0.9±1.2	10.9±6.6
Range	0.5-4.1	0.1-5.0	0.0 - 4.0	3.4-27.2	0.0-6.6	0.0-5.3	1.5-33.2
Requirement *	1.0	0.5	1.0	20	0.5	0.7	10-25
% do not comply with SLS requirement	36.7	40	50	83.3	83.3	53.3	36.7
% comply with SLS requirement	63.3	60	50	16.7	16.7	46.7	63.3
*Minimum as per SLS1635: 2019 and SLS 1634:2019							

Nitrogen is an essential constituent of chlorophyll and proteins in plants. Further, nitrogen is essential for vigorous growth, branching or tillering, leaf production, size enlargement, and yield formation of plants [35].

According to the findings (Table 4) average total nitrogen content in tested compost samples is 1.8±1.4 g/100g. From the tested samples, 36.7 % do not comply with the requirements of both the standards, SLS1635: 2019 and SLS 1634: 2019. In urea, the average total nitrogen content is 46.1±0.1 g/100g. When compared with the nitrogen content in urea, compost samples contain 26 times lower amounts of nitrogen.

Ammonium and nitrates ions are readily available for plant uptake just after the application of organic fertilizer, however, organic nitrogen has to be decomposed to simple and soluble organic forms, before it can be taken [36]. Although nitrogen is not phytotoxic at agronomic levels, elevated levels of ammonium can be phytotoxic, especially to seedlings. Moreover, the susceptibility of ammonium varies depending on the plant [37].To increase the nitrogen content in organic fertilizer, products of plants such as, tea leaves, wool, green plants, cottonseed meal (6% N), corn gluten (9% N), soybean meal (7% N) , animal by-products such as manure, and urine, blood meal derived from slaughterhouse waste, guano such as seabird guano (8 to 12% N) feather meal (14 to 16% N) a by-product of the poultry industry, fish meal (10 to 14% N) and fish emulsion (2 to 5% N) and seaweed fertilizers can be used to increase the nitrogen content in organic fertilizer since they contain higher nitrogen content [38,39].

The average phosphorous content of organic fertilizer is 1.7±1.4 g/100 g whereas the average phosphorous content of triple super phosphate is 47.6±0.7g/100 g. Hence, the availability of phosphorous in organic fertilizer is significantly lower than that of chemical fertilizer, triple super phosphate. From the tested compost samples, 40 % do not comply with the requirements of both the standards, SLS1635:2019 and SLS 1634:2019. However, the availability of phosphorus in composts compares favourably with that of conventional phosphorus fertilizers [40,41]. This is due to the fact that the elevated phosphorus levels in soil, which are caused as a result of the application of inorganic fertilizer, move off the field to pollute surface water [42]. To increase the phosphorus content of organic fertilizer, minerals such as rock phosphate and animal by-products such as fish wastes, and bone meal can be incorporated [43].

The average total potassium content of organic fertilizer is 1.7±1.4g/100 g whereas the average potassium content of MOP is 47.6±0.7 g/100g which is significantly ($p \leq 0.05$) lower than that of organic fertilizer. From the tested samples, 50 % do not comply with the requirements of both the standards, SLS1635: 2019 and SLS 1634:2019. Addition of banana peels, oak and fruit tree leaves, and wood ash helps to increase the potassium content in organic fertilizer [43].

According to the regulations of the European Union, solid organic fertilizer shall contain at least one of the primary nutrients: nitrogen (N), phosphorus pentoxide (P_2O_5) or potassium oxide (K_2O). If a solid organic fertilizer contains more than one declared primary nutrient, then it should contain at least 1 g/100g of total nitrogen (N), 1g/100g of total phosphorus pentoxide (P_2O_5), or 1 g/100g of total potassium oxide (K_2O). In addition to that, the sum of those nutrient contents shall be at least 4 g/100g.

According to the specifications in SLS1634: 2019 and SLS 1635:2019, organic fertilizer should contain at least 1g/100g of total nitrogen(N), 0.5 g/100g of total phosphorus pentoxide (P_2O_5), or 1 g/100g of total potassium oxide (K_2O). In SLS standards the minimum level of availability of phosphorus pentoxide

(P_2O_5) is lower than that of the regulations of the European Union although the minimum level for total nitrogen (N) and total potassium oxide (K_2O) are the same in both [10,11,44].

The average organic carbon content of compost samples is 13.2±6.7 g/100 g. According to specifications of SLS 1634: 2019 and SLS 1635:2019, the minimum organic carbon content of compost should be 20 g/100 g while organic carbon (C) content in a solid organic fertilizer shall be at least 15 g/100 g according to the regulations of the European Union [44]. Since the main source of soil organic matter is plant residues, it contains all the essential plant nutrients. Meanwhile, the stable organic fraction which is called humus adsorbs and holds nutrients that are in plant-available form. Additionally, organic matter act as an agent to improve soil structure, maintain tilth and minimize erosion [45].

The level of organic matter indicates the degree of decomposition of organic fertilizer. If the organic matter content is high in compost, it means that the compost may not have been thoroughly composted. This type of compost may contain unstable organic matter which might be lost as carbon dioxide gas as a result of rapid decomposition when the compost is applied to the field. In addition to that organic matter in compost is the main source of energy in the compost and also organic matter within the soil circulates nutrients [46].

Organic substances in the soil are important nutrient sources. Moreover, some substances cause the mobilization of nutrients from soil mineral reserves by producing organic acids, which dissolve minerals. Furthermore, these organic acids chelate substances excreted by roots and/or by microbes. For example, these chelates may bind iron from iron phosphate resulting in to liberate of phosphate anions [25]. Therefore, the application of organic fertilizer is beneficial for crops.

Organic matter involves in short-term fixation of nutrients such as nitrogen (N), phosphorous (P), and sulfur(S) into micro-organisms, leading to a transient deficiency at wide ratios of carbon with these elements such as C:N, C:P, and C:S ratio. Although long-term fixation of these elements into stable humid substances appears to be a loss, it becomes advantageous because of its positive effect on soil aggregation and on soil structure [25].

The C:N ratio provides an index of the quality of soil organic matter. In fertile soils, soil organic matter varies in the range of 10–15:1 [25]. Further, the carbon-to-nitrogen (C:N) ratio of compost is approximately proportional to overall energy contained in the compost. Composts with a higher C:N ratio immobilizes nitrogen for longer periods of time. However, immobilization is less significant in stable compost since soil microbes will metabolize the carbon in stable compost slowly. To contrast, composts that have low C:N ratio (C:N<20:1) gradually mineralize nitrogen with little or no immobilization (Bruunetal., 2006) [47]. More over carbon-to-nitrogen (C:N) ratio of compost helps to predict the source of origin. For an example, composts with lower C:N ratio (<10:1) are caused as a result of degradation of manures, bio solids, or food wastes.

When the plant materials are high, such compost will have a higher C:N ratio (>25:1) even after successful decomposing process, due to the presence of lignin that resists degradation. This kind of compost can be used as mulches to control weeds and conserve water. Mulch is applied to the soil surface to cover the soil surface. Furthermore, mulch is not used as a source of nitrogen for plants. Due to elevated C:N ratios (>40:1), the nutrients available to weeds are limited in these composts. Therefore, compost with higher C:N ratios can be used to fulfil this requirement. More over composts with low C:N ratios are ideal to use as soil amendments. Soil amendments mix into the root zone where nutrient availability is crucial for the growth and development of crops [42].

According to findings, based on the analysis of 20 compost samples of Sri Lanka which were municipal solid waste origin, from the 17 characteristics analyzed, average of 78±9% of characteristics comply with the requirements of SLS 1634:2019 standard. In addition to that, all the 17 characteristics of only 01 sample complied with requirements of SLS1634:2019. However, moisture, electrical conductivity, C:N ratio, Potassium oxide, and Magnesium were the parameters those did not comply with SLS 1634:2019. In the tested samples, nitrogen, phosphorous and potassium contents varied substantially. But all the tested samples comply with SLS 1634:2019 for the specifications given for heavy metals [48].

The website of Department of Agriculture Sri Lanka, recommends the amount of urea, TSP, and MOP to be added to various crops based on growth stage, geographical area, and irrigation condition. Based on average nitrogen, phosphorous, and potassium content of these chemical fertilizer and compost samples, the required amount of compost quantity to supply recommended nitrogen, phosphorous, and potassium content by the Department of Agriculture was calculated. However, as a rule of thumb, the Department of Agriculture recommended to apply 10-12 metric tons of organic fertilizer per hectare. According to the calculations, based on the findings on the average nitrogen, phosphorous, and potassium content of organic fertilizer samples analyzed, the application of organic fertilizers 10 metric tons will supply 130 kg of nitrogen, 120 kg of phosphorous, and 110 kg of potassium per hectare. Table 5 depicts the recommended urea, TSP, and MOP composition for several crops by the Department of Agriculture and the required amount of organic fertilizer to supply the required nitrogen, phosphorous, and potassium contents [49,50,51,52]

Table 5. Recommended urea, TSP, and MOP composition for several crops, nitrogen, phosphorous, and potassium content supplied via urea, TSP, and MOP and organic fertilizer.

To calculate nutrient requirements in table, Urea N percent by mass is 46.1, TSP, P percent by mass is 20.8, MOP, K percent by mass is 50.7 In organic fertilizer, fresh basis N % is 1.3, fresh Basis P % is 1.2 and fresh basis K %1.1										
Based on recommendations by department of agriculture, Sri Lanka										
Crop	**Time of application with respective to date of planting**	**Recommended amount of chemical fertilizer (kg per hectare)**			**Amount of N in Urea, P in TSP and K in MOP (kg per hectare)**			**Required amount of organic fertilizer (kg per hectare) to supply**		
		Urea	**TSP**	**MOP**	**N**	**P**	**K**	**N**	**P**	**K**
Big onions	before 1 or 2 days	65	100	50	30	21	25	2305	1732	2304
	after 3 weeks	65	–	–	–	–	–	2305	–	–
	after 6 weeks	65	–	25	–	–	–	2305	–	1152
Maize*	Basal fertilizer	75	100	50	35	21	25	2660	1732	2305
	4 to 5 weeks	240	–	–	111	–	–	8511	–	–
Maize **	Basal fertilizer	75	100	50	35	21	25	2660	1732	2305
Finger millet	Basal fertilizer	65	55	75	30	11	38	2305	953	3457
Soy bean	Basal fertilizer	50	100	75	23	21	38	1773	1732	3457
Potatoes	Basal fertilizer	55	270	125	25	56	63	1950	4676	5761
	# 2 weeks	110	–	–	51	–	–	3901	–	–
	# 3-4 weeks	165	–	125	76	–	63	5851	–	5761
Peanuts	Basal fertilizer	35	100	75	16	21	38	1241	1732	3457
	at flowering stage	30	–	–	14	–	–	1064	–	–
Cabbage	Basal fertilizer	110	270	75	51	56	38	3901	4676	3457
	After 3 weeks	110	–	75	51	–	38	3901	–	3457

	After 6 weeks	110	–	75	51	–	38	3901	–	3457
Okra	Basal fertilizer	50	195	25	23	41	13	1773	3377	1152
	# 2 weeks	50	–	25	23	–	13	1773	–	1152
	#5 weeks	100	–	50	46	–	25	3546	–	2305
	# 8 weeks	100	–	50	46	–	25	3546	–	2305
Paddy ***	*******	225	55	60	104	11	30	7979	953	2766
Paddy****	*******	175	35	50	81	7	25	6206	606	2305
Paddy*****	*******	140	35	50	65	7	25	4965	606	2305
Paddy ******	*******	100	55	110	46	11	56	3546	953	5070

*rainfed conditions
**under irrigation
***for paddy fields cultivated under irrigated conditions Intermediate zone and dry zone
****for paddy fields cultivated under rainfed conditions Intermediate zone and dry zone
*****for paddy fields cultivated under irrigated conditions Wet zone
******for paddy fields cultivated under rainfed conditions Wet zone
*******3,3.5 and 4 months old (Total)
#Top dressing

According to table 05, to supply the required amount of nitrogen, phosphorous, and potassium, the required amount of organic fertilizer is significantly higher than that of chemical fertilizer to these crops as per the guidelines of the department of agriculture Sri Lanka.

IV. CONCLUSION AND RECOMMENDATION

All the tested compost samples comply with specifications given for heavy metals including lead, arsenic, chromium, cadmium, mercury, and nickel in Sri Lanka standards for organic fertilizer, SLS1635:2019, and SLS1634:2019 although nearly half of the samples do not comply with SLS specifications for pH, electrical conductivity, moisture, content of sand, total nitrogen, phosphorous, potassium, calcium, magnesium, C:N ratio, and organic carbon. Therefore, it is important to develop a mechanism to pricing of compost based on nutritional quality. Since the nutritional quality of compost can vary from batch to batch, it is essential to analyze nutritional quality of each batch prior to pricing. Based on the analytical results on nutrient composition, and nutrient requirement of crops front pack labelling of crop recommendations of commercially available compost will help to maximize return on investment to customers.

The amount of compost to be added per hectare to fulfil the nutrient

requirement according to the recommendations of the Department of Agriculture of Sri Lanka, is significantly high. Therefore, it is essential to promote and facilitate large-scale compost production. Establishing a proper regulatory mechanism to improve the quality of organic fertilizer in terms of increasing nutrient content, via minimizing moisture and sand content will help to reduce the quantity of organic fertilizer to be applied per hectare to fulfil the nutrient requirements of crops. Since inter-sample nutrient composition of tested samples varied significantly, it can be concluded that during the process of compost production, the nature and the quality of materials used would vary. Hence it is recommended to establish a tailor-made set of regulations and quality standards for composting process and to produce compost targeted for different purposes such as organic fertilizer, soil fertility enhancer, landfill material, fertilizer for reclamation sites, soil amendments, or as mulches for different crops and conditions, etc. after identification of common core standards and regulations across required applications and specific conditions of other countries.

Since compost alone cannot cater to the demand for fertilizer requirement it is recommended to carry out, regulate, monitor, and evaluate organic farming and to sell the harvest separately while integrated farming is used in mass-scale agriculture to prevent a shortage in the harvest.

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Insights into the *Aspergillus* bio fertilizers – Potential and Prospects

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Abstract: This article is a bibliometric analysis of *Aspergillus* bio fertilizers carried out from 2010 to 2022. The purpose of this article is to identify the potential and prospects of *Aspergillus* bio fertilizers to make a foundation for future research. The analysis is based on 36 research articles identified using Google scholar search engine. There is an increasing interest in *Aspergillus* bio fertilizers related research over the study period. It was able to identify *Aspergillus niger* as the most potent species for the production of *Aspergillus* bio fertilizers at commercial level. Among the minerals that have low bioavailability, phosphorus (P) is the mineral that can be solubilized successfully by the *Aspergillus* bio fertilizers. When expanding the studies related to *Aspergillus* bio fertilizers to a commercial level, liquid and solid formulations are the most suitable formulation types in the production of *Aspergillus* biofertilizers. The majority of considered studies have concentrated in the Asiatic region and the path is still open for the investigation of efficient *Aspergillus* bio fertilizers to mobilize essential micronutrients. The findings of this paper can act as a useful foundation and reference for the researchers and provide insights for directing future research on *Aspergillus* bio fertilizers.

Keywords: ***Aspergillus*, Bio fertilizers, Fungi, Minerals, Plant**

I. INTRODUCTION

The current agricultural sector faces challenges to fulfil the increasing food demand due to shortage of arable land and rapid increment of world population. Conventional farming technologies contribute to fulfil the food demand, but they have adverse effect to humans and environmental sustainability. The crop productivity along with agro-environmental sustainability able to be achieved by encouraging farmers to use agro-environmental green products such as bio fertilizers incorporating of live microbes instead of chemical-based inputs. The eco-friendly products play a significant role in plant growth promotion, crop productivity and regenerating soil fertility and texture sustainably. Mineral solubilizing microorganisms such as fungus and

bacteria that provide huge amounts of nutrients like phosphorus, potassium, zinc and selenium are essential for plant growth and crop production and have a potential to develop as bio fertilizers. *Aspergillus* genus belong to kingdom fungi, phylum Ascomycota has a huge potential to solubilize minerals by producing beneficial metabolites and to facilitate atmospheric N fixation through symbiosis. Production of organic acids, siderophores, exopolysaccharides and enzymes by *Aspergillus* species involves solubilizing minerals and symbiotic fixation of atmospheric N [1].

In this paper, studies related to *Aspergillus* bio fertilizers within the time period of 2010-2022 have analyzed and visualized the potential and prospects.

II. Materials and Methods

A literature survey on *Aspergillus* bio fertilizers for bibliometric analysis was done using the Google Scholar in October 2022. The journal articles and full papers of conference proceedings published in English between 2010 and 2022 were selected for the analysis. "*Aspergillus* bio fertilizers" was used as the keyword to search publications, which resulted in 42 studies. Published year of publication, type of *Aspergillus* species used, minerals; make available to plants by *Aspergillus* sp., type of the bio fertilizer, carrier material used, plant varieties used for testing the application, country of the study was carried out and the reference of all publications were recorded. Only 36 publications which containing above details are considered for the analysis. Publication intensity and its trend regarding *Aspergillus* bio fertilizer studies was analyzed. The trending and potent *Aspergillus* species to use as bio fertilizers, minerals that can be converted into bioavailable form by *Aspergillus* species and type of biofertilizers that can be formulated by using *Aspergillus* species were also analyzed.

III RESULTS AND DISCUSSION

Summarization of identified 36 publications was carried out using a tabular format including the information like the *Aspergillus* species used for the study, type of the bio fertilizer formulated, carrier material used in the formulation, plant varieties used for testing the application, country where the study was carried out and the reference of the publication (Table 01).

A. The volume of publications on Aspergillus bio fertilizers

Even the number of published articles shows fluctuations with the time, it shows an increasing trend and shows the increasing importance of the *Aspergillus* bio fertilizers over the years (Figure 1). Among all publications, 75% have published in the latter half of the period considered for the analysis. Increasing number of *Aspergillus* bio fertilizer research, which also reflects the increasing concerns over environmental impacts of the usage of conventional chemical fertilizers and the huge potential of *Aspergillus* species to use as bio fertilizer. During the period of 2010 - 2022, the highest number of publications were recorded in 2019, 2021, 2022 (Up to October) and world chemical fertilizer production is at a

constant level from 2016 to 2022 [2] while world bio fertilizer market has increased within relevant time [3].

B. Analysis of Aspergillus species used for bio fertilizer studies

Among the analyzed 36 publications, 66.67% studies have used *Aspergillus niger* in bio fertilizer studies (Figure 2). According to Visser et al., 1997, micromorphology, best fitness to grow and survival and vital ability to solubilize minerals are the significant beneficial characteristics of *A. niger* that caused to use it more frequently in bio fertilizer production [4].

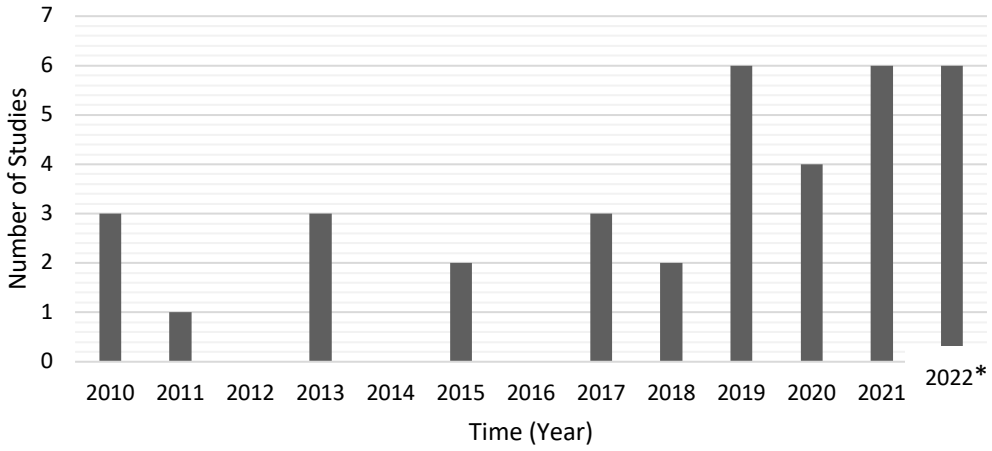
C. Analysis of the minerals; make available to plants by Aspergillus sp.

The highest number (86.11%) of identified publications have mentioned that *Aspergillus* bio fertilizers used to increase the bioavailability of P. Furthermore, *Aspergillus* bio fertilizers able to be used in enhancing K solubilization and N availability–to the plants. In addition to that, *Aspergillus* bio fertilizers chelate the heavy metals like Cr and Cd to lesser the toxicity to plants (Figure 3).

D. Analysis of the type of formulations used in Aspergillus bio fertilizers production

If there are four types of bio fertilizers as solid, liquid, granules and freeze-dried powders, *Aspergillus* bio fertilizers commonly formulated as solid and liquid bio fertilizers. Feasibility for the formulation, storage, delivery and application; *Aspergillus* bio fertilizers has focused to formulate as liquids and solids.

Different types of carrier materials such as culture media, organic materials (waste and under utilized materials etc.), fertilizers (organic and inorganic) (Table 01.) have used in the formulation of *Aspergillus* bio fertilizers due to the availability, compatibility, cost, toxicity etc. need to be considered in formulation. Optimization need to be done in selection procedure to select proper carrier material for formulation of efficient biofertilizer. There is a vital potential to use *Aspergillus* bio fertilizers for different type of crop varieties (Table 01). According to the selected publications, studies related to *Aspergillus* bio fertilizers are concentrated in Asian countries.



2022*- asterisk indicates data are till October 2022

Figure 1. The graph of number of publications Vs year

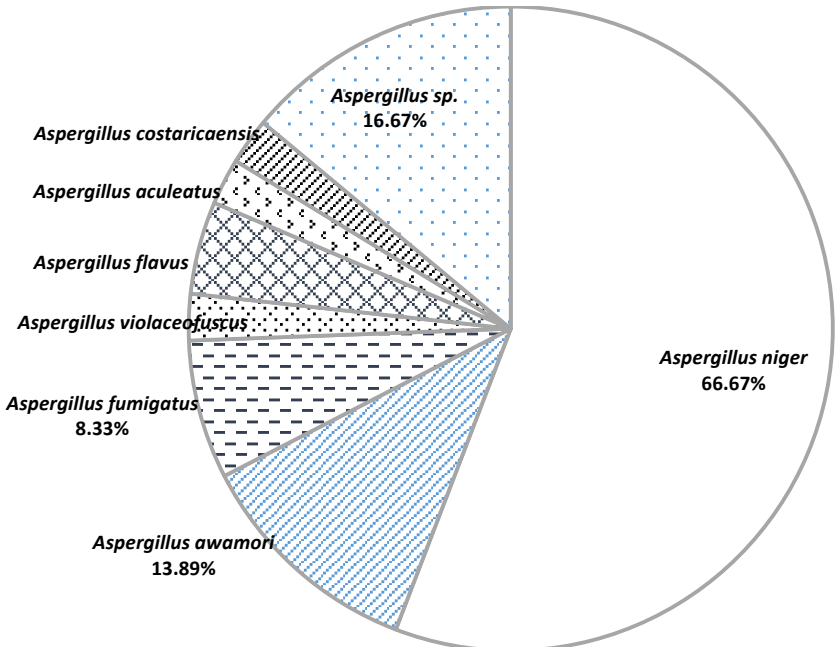


Figure 2. The graph of reported *Aspergillus* species used for bio fertilizer studies, 2010-2022

Table 01. Summarization of identified publications

Species	**Minerals, make available to plants by *Aspergillus* sp.**	**Biofertilizer**		**Used plant variety for application trial**	**Country**	**Reference**
		Type	**Carrier Material**			
Aspergillus awamori, *Aspergillus niger*	P, Zn	Solid	Vermicompost	American Maximum Yellow F1 hybrid marigold	India	[5]
Aspergillus sp.	N,P,K	Liquid	Fermented soybean liquid waste and coconut water waste	Spinach (*Amaranthus tricolor L.*)	Indonesia	[6]
Aspergillus niger	Chealte Cr and Cd	Liquid	Culture filtratre of fungi grown in Czapek broth	Tomato (*Solanum lycopersicum* L.)	Pakistan	[7]
Aspergillus sp.	P	Liquid	Grown in Pikovskaya's liquid broth	Maize (*Zea mays*)	India	[8]

Aspergillus awamori, *Aspergillus niger,* *Aspergillus fumigatus*	P , N	Liquid	Grown in Pikovskaya's liquid broth	Pigeon pea (*Cajanus cajan*)	India	[9]
Aspergillus niger	N, P, K	Liquid	Coconut water waste	Chili, Tomato, Eggplant	Indonesia	[10]
Aspergillus niger	P	Liquid	Culture filtrate (CF) or conidial suspension (CS)	Wheat	Egypt	[11]
Aspergillus niger	P	Liquid	Spore suspension	Wheat	Iraq	[12]
Aspergillus niger	N and P	Liquid	Wheat bran + water	Bottle gourd (*Lagenaria siceraria*) Okra (*Abelmoschus esculentus*)	Pakistan	[13]
Aspergillus awamori	N and P	Solid	Sterile lignite	Rice	India	[14]
Aspergillus niger	P	Liquid Solid	2% molase +NH_4Cl of 0.05% broth medium Mixture of compost and peat	Maize (*Zea mays*)	Indonesia	[15]
Aspergillus niger	N and P	Solid	Urea, P2O5	Celery	Indonesia	[16]

Aspergillus fumigatus, *Aspergillus niger*	N and P	Liquid	PVK Broth	Chilli	India	[17]
Aspergillus niger	P	Liquid	Sterile distilled water	Common bean	Brazil	[18]
Aspergillus violaceofuscus	Chealte Cr and Cd	Solid	Fresh biomass	Okra	Pakistan	[19]
Aspergillus niger	P	Solid	Peat, corn cob mixed with 20% perlite (CCP), wheat husk mixed with 20% perlite (WHP), and composted cattle manure mixed with 20% perlite (CCMP)	Chinese cabbage	China	[20]
Aspergillus niger, *Aspergillus favus*	N, P, K	Solid	Bio-organic extracts from plants, animals and fish liquids with the addition of molasses and compost	Sun flower crop (*Helianthus annuus*)	Pakistan	[21]
Aspergillus niger	N, P, K	Solid	Saw dust and the cow dung and or poultry	Cowpea (*Vigna unguiculata*)	Nigeria	[22]

			droppings mixed in a ratio of 30:1			
Aspergillus niger	Zn, Mn, Cu, S	Solid	ZnO Mineral, MnO Mineral, and CuO Mineral and S in starch composites formulations	*Lolium multiforum Lam.*	Brazil	[23]
Aspergillus aculeatus	K	Solid	Sterile mixture of sawdust and sand	Perennial ryegrass	China	[24]
Aspergillus flavus	P, K, Zn	Liquid	Pikovskaya broth	*Zea mays*	Nigeria	[25]
Aspergillus fumigatu, *Aspergillus niger*	P	Solid	Cassava starch (1%), poultry droppings (3%) and Ground cassava peel (96%)	Pigeon pea (*Cajanus cajan*)	Nigeria	[26]
Aspergillus awamori	P	Solid	P_2O_5	Mung bean (*Vigna radiata*)	India	[27]
Aspergillus niger, *Aspergillus awamori*	N, P, K	Liquid	Sugar Press Mud (SPM)	Chick pea, Wheat, Fenu greek, Red chilli pepper	India	[28]

Aspergillus niger	N,P,K	Solid	poultry waste enriched with molasses and algae	Barley	Morocco	[29]
Aspergillus niger	P, K	Liquid Solid/Granular	Conidial suspension with sterile water Dried conidial spores mixed with wheat flour, corn starch, granulated sugar and deionized water	Lettuce, Kale, Scarlet eggplant, Watermelon, Melon, Pepper, Tomato	Brazil	[30]
Aspergillus niger	P, K, Zn	Solid	Press mud	*Zea mays*	Pakistan	[31]
Aspergillus sp.	N	Liquid	Sterile Distilled water	Rice	Sri Lanka	[32]
Aspergillus sp.	P	Liquid	Chopped peels of banana, papaya and carrot	Brinjal	India	[33]
Aspergillus niger	N, P, K	Solid	N,P,K, Fertilizers	Chilli	India	[34]
Aspergillus sp.	N,P	Solid	Chemical Fertilizer	Strawberry	Sri Lanka	[35]
Aspergillus sp.	N,P	Solid	Chemical Fertilizer	Strawberry	Sri Lanka	[36]
Aspergillus niger	P, K	Liquid	Water	Maize (*Zea mays*)	Indoneia	[37]

Aspergillus niger	P	Solid	Compost + 10% NPK and 10% guano fertilizer	Rice	Indonesia	[38]
Aspergillus costaricaensis	P, K	Solid	Peat	Maize (*Zea mays*)	Indonesia	[39]
Aspergillus niger	N,P,K	Solid	Commercial fertilizer	Peanut	China	[40]

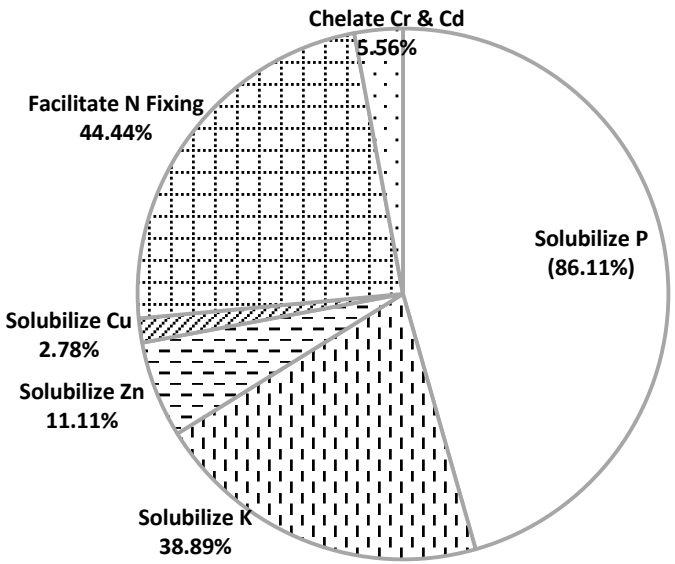


Figure 3. The graph of Type of minerals, make available to plants by *Aspergillus* biofertilizers

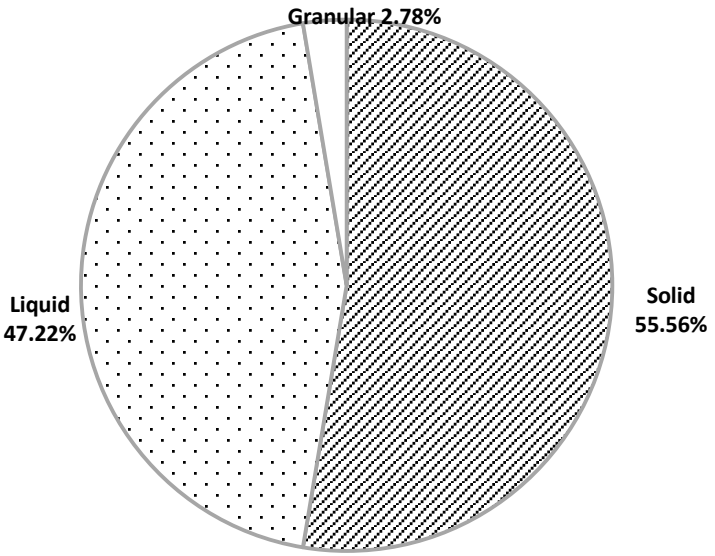


Figure 4. The graph of type of bio fertilizers used in *Aspergillus* bio fertilizer formulations

IV. CONCLUSIONS AND RECOMMENDATIONS

The analysis of published 36 studies within the considered time period (2010-2022) shows positive trend in the field of *Aspergillus* bio fertilizers related studies. The current trend to shifting into sustainable agriculture by using the potential of *Aspergillus* species as bio fertilizers is globally used as a green option. *Aspergillus niger* is a frequently used fungus which have the huge potential to solubilize minerals essential for plants. *Aspergillus* species can successfully use to increase the bioavailability of P among the minerals, which have low solubility. There is a wide range of crop varieties that can be targeted by this *Aspergillus* bio fertilizers industry at the commercial level. According to the selected publications, solid and liquid types are the most suitable formulation types to produce *Aspergillus* bio fertilizers commercially. Further studies are required to improve and establish this state of art technology to reach the development of the economy. The findings of this paper will make a foundation for the development of *Aspergillus* bio fertilizers to elaborate this to a commercial level.

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FOCUS AREA

Health

COMPARATIVE EVALUATION OF QUALITY OF DIFFERENT BRANDS OF AMOXICILLIN CAPSULES AVAILABLE IN COMMUNITY PHARMACIES IN JAFFNA, SRI LANKA

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Abstract: Amoxicillin is a semisynthetic, β lactam antibiotic that is extensively prescribed among antibiotics. The effectiveness of drug therapy can be changed according to the amount of active ingredients in the dosage forms. Lack of quality may lead to toxicity or failure of therapeutic goals and may develop resistance. Therefore, this study aims to evaluate and compare the quality of different brands of Amoxicillin capsules with innovator brand purchased from private pharmacies in the Jaffna municipal area. Commonly available 7 brands of Amoxicillin 250mg capsules were collected from community pharmacies of the Jaffna municipal area and coded from A_1 to A_7. Qualities of those brands were determined by using the methods such as weight variation, dissolution, and uniformity of content tests as described in British Pharmacopoeia (BP). The dissolution profile data of seven brands were compared with the innovator brand (Amoxil) using Oneway ANOVA followed by the Dunnett T3 test.

All the tested brands complied with the official specifications for weight variation and dissolution tests. All brands of Amoxicillin Trihydrate capsules complied with the specified BP limits except brand A4 which failed to release the stated amount (89.9 % ±0.15). The statistical comparison for the drug release reported that some of the brands showed significant differences ($p < 0.05$), indicating that the *in vitro* drug release would affect their *in vivo* bioavailability. Authorities of the country should take more attention to the quality of antibiotics, especially imported drugs.

Keywords: Amoxicillin capsules, Quality, Evaluation, Sri Lanka

I. INTRODUCTION

Amoxicillin is a semisynthetic β–lactam antibiotic with a broad spectrum bactericidal activity against many gram-positive and gram-negative microorganisms. Amoxicillin interferes with the synthesis of peptidoglycan in the bacterial cell wall. They inhibit trans peptidation enzyme which crosslinks the peptide chains of the peptidoglycan soon after they attach to the binding site of bacteria [1]. There are 13 generic oral solid products of amoxicillin registered with the National Medicines Regulatory Authority (NMRA). These include locally manufactured and imported brands including the innovator product [2].

According to British Pharmacopeia (BP), Amoxicillin capsules quality is evaluated using tests such as uniformity of weight, uniformity of content, and dissolution test.

A generic drug is a medication that has the same active ingredient as the innovator and yields the same therapeutic effect. The effectiveness of drug therapy can be altered by the amount of active ingredients. When there is a lack of quality it may lead to the failure of therapeutic goals, thereby the development of resistance.

Care should be taken to avoid the change in composition among different brands due to the development of resistance. As most of the drugs are imported into Sri Lanka, the quality of antibiotics should be strictly monitored.

Therefore, this study is aimed to determine the pharmaceutical quality of amoxicillin capsules and compare them with the innovative brand.

II. MATERIALS AND METHODS

Materials and reagents

Chemicals and reagents used to experiment were 250mg Amoxicillin Trihydrate capsules, *Amoxicillin Trihydrate*, *Cefadroxil* BPCRS, Acetonitrile, *Potassium dihydrogen orthophosphate, Sodium hydroxide*, Absolute ethanol, Ether.

Equipment

The Equipment used for the experiment was an Electronic analytical balance, HPLC, United State Pharmacopeia (USP) 2 (paddle type) Dissolution test apparatus, Ultra-Violet (UV) Visible Spectrophotometer, pH meter, and Thermometer.

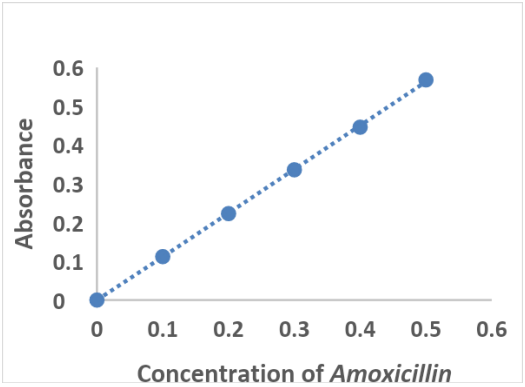

Figure 1. Calibration curve for Amoxicillin Trihydrate

Methods

This laboratory-based analytical study was carried out in State Pharmaceutical Manufacturing Corporation (SPMC), Sri Lanka. All the brands of Amoxicillin Trihydrate capsules available at community pharmacies of the Jaffna municipal area were identified by a mini survey. The pharmacies which had air-conditioning facilities for the storage of drugs were selected for the study. A sample of 50 capsules was purchased from the same batch while checking properly for their manufacturing license number, batch number, manufacturing, and expiry dates before purchasing[4]. Batches that are near to expiry date were excluded (at least a three months gap should be there). The detailed descriptions of these products are presented in table 1.

Calibration curve

The calibration curve of Amoxicillin trihydrate was constructed as shown in Figure 1 using the following procedure. Stock solution for amoxicillin trihydrate was prepared by dissolving 100 mg of amoxicillin in 100 ml of distilled water in the volumetric

flask with shaking till the drug is completely dissolved. The stock solution of 1 ml was diluted into 100 ml of distilled water. The further Stock solution was diluted with distilled water to prepare different concentrations of drug solutions by serial dilutions. Then the drug solutions were analyzed spectrophotometrically at 254 nm using distilled water as blank.

Methods

A. Uniformity of Weight

Twenty (20) capsules from each brand were selected randomly and weighed individually. The average weight of each capsule and the percentage deviation for each brand was determined [5].

B. Uniformity of Content

The uniformity of content of each brand is carried out according to BP (2017) by HPLC [6]. This test was replicated four times for each brand and the average area for the chromatogram was taken to calculate the mean assay percentage.

The reverse-phase chromatography was used. Mobile phase A and Mobile phase B consisted of *Acetonitrile* and 25% v/v solution of 0.2 M *potassium dihydrogen orthophosphate* at 1:99 and 20:80 volume ratios respectively with pH adjusted to 5.0 with 2 M *sodium hydroxide*. A flow rate of 1ml/min was employed with an injection volume of 50µl of each solution at < 60 $^{\circ}$C temperature.

Mobile phase A with a volume of 80 ml was added to a quantity of the mixed contents of 20 capsules containing an equivalent of 60mg of Amoxicillin and shaken for 15 minutes. The content was mixed with the aid of Ultrasound for 1 minute. Sufficient mobile phase A was added to produce 100ml. The content was mixed and filtered by using Whatman GF/C filter paper. Then it was analysed by HPLC.

Table 1. Different brands of Amoxicillin capsules

Code	Batch number	Country
A_1	PTG15	Sri Lanka
A_2	18AX15	Sri Lanka
A_3	AA10381	India
A_4	MXCC1904	India
A_5	PXABV0036	Bangalore
A_6	S364190101	India
A_7 (Amoxil)	LOTWP2P	Sri Lanka

Dissolution test

The dissolution test was performed by using USP 2(paddle type) operated at 75rpm for 60 minutes using 900ml of water maintained at a temperature of 37 ±0.5 ^{0}C. The test was performed 12 times for each brand. Six (6) capsules were placed in each reciprocating glass cylinder and the sample solution was collected at intervals of 15, 30, 45 & 60 minutes. A volume of 10ml of the sample solution was collected at every time interval and immediately replaced 10ml of fresh distilled water. Each collected sample solution was filtered by using a filter with a porosity of 0.45µm. The filtrated solution with a volume of 10ml was diluted to 100ml with water. Then 10ml from the resulting diluted solution was diluted again to 100ml with water. The amount of dissolved Amoxicillin was determined by measuring the absorbance value at a wavelength of

maximum absorbance at 254 nm using UV-Visible Spectrometer[1].

Statistical analysis

Results obtained were presented as means with standard deviations. The data were computed and analyzed using SPSS (Statistical Package of Social Science) 25. One-way ANOVA with Dunnett T3 post hoc test was conducted to compare the quality of different brands of Amoxicillin capsules with the innovator brand at a 95% confidence interval. 30 minutes time interval of dissolution time was used for comparison between the brands. A *p*-value less than 0.05 was considered a statistically significant difference.

III. RESULTS AND DISCUSSION

A. Uniformity of Weight

The weight variation standard shows that the recommended procedures were followed to ensure uniformity of distribution of the drug in capsules.

The results on the uniformity of weight as represented in table 2 revealed that all the capsule weights complied with BP, as not more than two of their weights deviated from the average weight by more than the percentage deviation of 7.5 and none deviated by more than twice that percentage.

A similar study conducted in Kenya reported that all the Amoxicillin capsules except three brands passed the weight variation test [7]. While a study done in Brazil [8] also showed that three out of thirteen brands were found to be disapproved on average weight assay. It might be due to the less amount of content in the capsules resulting in a poor manufacturing process.

This test ensures that the drug is distributed in a narrow range around the active ingredient to produce a consistent and correct dosage. [9].

B. Uniformity of Content

The uniformity of content of 7 brands was evaluated by the HPLC method. The assay test was done three times for each brand and the average area for the chromatogram was used to determine the mean assay percentage.

Table 2. Mean Assay % of all seven brands of Amoxicillin capsules

Code	Mean assay%
A1	103.38 ±0.05
A2	100.41 ±0.52
A3	105.59 ±0.18
A4	89.9 ±0.15
A5	103.54 ±0.11
A6	98.54 ±0.41
A7	98.67 ±0.02

Table 2 represents that all the Amoxicillin capsules except A4 met the specified BP limits where the amount of active ingredient released should be in the range of 92.5- 110%.

A study on amoxicillin formulations conducted in Ghana, Nigeria and the United Kingdom[3] declared that 19 out of 20 amoxicillin capsules tested complied with USP tolerance limits. A relative study done in Mexico declared that eight of the studied ten brands have passed the content uniformity test with the HPLC method[6].

This failure could be due to low active ingredients or poor formulation and processing. Although all the brands have desired quality at the time of manufacturing

poor storage conditions and transport conditions may affect the quality of drugs.

C. Dissolution test

Table 3 represents the results of the dissolution test that was done on 12 capsules for each brand at four-time points (15, 30, 45, and 60 minutes).

Table 3. Drug release % of different brands of Amoxicillin Capsules

Code	Percentage drug release			
	15min	**30min**	**45min**	**60min**
A1	63.81 ±3.99	82.49 ±3.51	95.37 ±2.33	99.87 ±1.23
A2	80.72 ±4.2	97.00 ±0.92	103.25 ±0.98	104.04 ±1.24
A3	78.46 ±2.81	85.26 ±5.26	93.24 ±4.41	94.79 ±4.67
A4	67.53 ±3.84	84.29 ±3.48	92.56 ±2.82	94.18 ±2.57
A5	94.93 ±3.25	100.00 ±4.06	101.53 ±3.57	100.71 ±3.57
A6	92.87 ±3.12	100.05 ±4.15	101.8 ±3.08	103.52 ±2.3
A7	62.28 ±2.99	82.83 ±5.00	90.25 ±6.12	95.37 ±5.49

Data represented as mean ± SE (n=12)

Drug dissolution is a vital condition required for drug absorption into the body where it directly relates to bioavailability[10]. This acts as a key factor that ensures the release of a batch which thereby determines the batch quality and the constant quality of the product throughout its shelf life[11].

According to the British pharmacopoeia specifications, the drug should be released by more than 80% within 60 minutes. All the brands complied with the BP limit for the dissolution test as shown in figure 2. A study conducted in Brazil[8] with 13 different brands and also similar studies conducted in Ghana, Nigeria and the United Kingdom explored that all brands passed the dissolution test[3].

A study done in Bangladesh to study 20 national and 4 multinational brands of Amoxicillin Trihydrate capsules reported that except for two national brands, the rest of the 24 brands comply with the pharmacopoeia specifications for the in vitro drug release[1].

Figure 2 represents the pattern of in vitro drug release of amoxicillin brands at different time points.

Statistical analysis

There was a significant difference ($p < 0.05$) in drug release at 30 minutes time point was observed between the innovator brand and other different brands of Amoxicillin Trihydrate capsules except A1, A3 and A4 using One-Way ANOVA with Dunnett T3 post hoc test as shown in table 4. This could be because of the varied GMP processes followed by different manufacturers for their brands[12].

Table 4. *p* values of Amoxicillin brands with innovator brand

Code	Significance value
A1	1.000
A2	0.000*

A3	0.996
A4	1.000
A5	0.000*
A6	0.000*

**significant difference at p-value < 0.05*

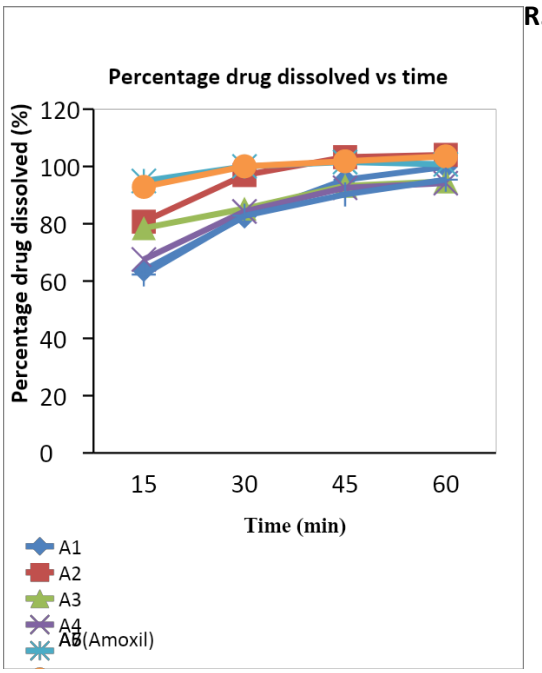

Figure 2. Drug Release % for different brands of Amoxicillin capsules

IV. Conclusion and Recommendation

This study revealed that all the brands of Amoxicillin Trihydrate capsules except A4 complied with the official specification of BP. Statistical comparison for the *in vitro* drug release showed that some of them have significant differences indicating that it might affect the *in vivo* bioavailability and the bioequivalence of the products.

Authorities of the country should take more attention to the quality of antibiotics especially imported drugs.

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VARIATIONS IN PATIENT DOSES FROM LUMBAR SPINE X-RAY EXAMINATIONS IN TWO PUBLIC HOSPITALS IN SRI LANKA

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Abstract: The lumbar spine X-ray is considered as a routinely performed radiographic investigation to diagnose various clinical indications, including lower back pain, fractures, arthritis, spondylolisthesis, tumors, and degenerative pathologies. On the other hand, lumbar spine X-ray examinations are typically associated with higher patient doses compared to all other projection radiographic examination types. These higher patient doses may increase the risk of stochastic and deterministic effects on patients.

Therefore, it is important to investigate the variability of patient doses in such procedures to reduce excessive radiation doses that do not contribute significantly to the clinical outcome of the examinations. The present study aimed to investigate the variability of patient doses in terms of kerma-area product (KAP) associated with lumbar spine anteroposterior (AP) and lateral (LAT) X-ray examinations performed in two public hospitals in Sri Lanka. This dosimetric study was conducted on two digital radiography (DR) systems in two public hospitals with 164 adult patients. The ages of the patients involved were from 18 years to 84 years, and their weights ranged from 34.2 kg to 87.6 kg. For each examination,patient-specific parameters (age, sex, and weight) and radiographic exposure parameters (tube voltage, tube current-exposure time product) were obtained. Likewise, the KAP values were measured using a KAP meter for complete examinations. Descriptive statistics were utilized for data analysis. In this study, patient doses obtained for the lumbar spine AP and LAT examinations showed high variability, evidenced by a larger range and high standard deviation (SD) values: 0.29-1.64 (SD=0.29) and 0.61-3.03 (SD=0.59) $Gy.cm^2$ for lumbar spine AP (hospitals A and B) and 0.73-3.90 (SD=0.77) and 0.84-2.49 (SD=0.41) $Gy.cm^2$ (hospitals A and B), respectively. The results of this study emphasize the need to establish reference dose levels for X-ray examinations performed in hospitals in order to optimize patient doses and improve current radiology practice.

Keywords: Kerma-Area Product, Patient Dosimetry, Radiation Protection, Lumbar Spine X-ray, Dose Optimization

I. INTRODUCTION

Worldwide, the number of projection X-ray examinations performed is increasing rapidly due to their widespread use in the initial diagnosis of diseases and injuries in patients. Besides its benefits, radiosensitive organs are also exposed to radiation doses, which may impose the risks of stochastic and deterministic (tissue reactions) effects on patients [1]. The lumbar spine is in the lower back region of the spine (L_1-L_5), which is identified as a common site of injuries [2]. Therefore, the lumbar spine X-ray is considered as the most routinely performed radiographic examination for the initial diagnosis of various clinical indications such as lower back pain, fractures, arthritis, spondylolisthesis, tumors, and degenerative pathologies [2]. In practice, two standard projections, anterior-posterior (AP) and lateral (LAT), are performed to evaluate lumbar alignment, vertebral body and disc space size, bone space and architecture, and the gross evaluation of soft tissue structures [3]. Generally, lumbar spine X-ray examinations are associated with relatively higher patient doses compared to all other projection radiographic examination types and contribute a large proportion of the collective dose [4]. For this reason, it is necessary to investigate the variability of patient doses in lumbar spine X-ray examinations to minimize dose variations in order to protect radiosensitive organs in the lumbar region (stomach, colon, and reproductive organs) [1].

Patient dosimetry is an important aspect of dose management for patients undergoing projection X-ray examinations. The purpose of patient dosimetry is to ensure that an as low as reasonably achievable (ALARA) dose is delivered to patients during radiographic procedures [5]. Also, it helps to minimize or avoid excessive radiation doses delivered to the patient that do not significantly contribute to the clinical outcome of radiographic examinations [6]. Patient doses can be estimated in terms of the kerma-area product (KAP), also known as the dose-area product (DAP), for the complete examination in projection radiographic examinations [7]. The KAP is considered as an appropriate dose quantity to measure the total amount of radiation delivered to the patient [7]. In recent years, dosimetric studies have been carried out for X-ray examinations in many countries to assess patient doses in order to improve patient protection. In the literature, there are few published dosimetric studies in Sri Lanka to date for X-ray examinations. Therefore, the results of this study will provide a useful review of the variability of patient doses for lumbar spine X-ray examinations carried out in two public hospitals. Furthermore, the findings of this study will be useful in exploring the possibility of further patient dose reductions. Therefore, the

present study aimed to investigate the variability of patient doses in terms of kerma-area product (KAP) associated with lumbar spine AP and LAT examinations performed in two public hospitals.

II. MATERIALS AND METHODS

This dosimetric study was conducted on two digital radiography (DR) systems (manufactured by SHIMADZU Cooperation) in two public hospitals in Sri Lanka with 164 adult patients. The ages of the patients involved were from 18 years to 84 years, and their weights ranged from 34.2 kg to 87.6 kg. The hospitals involved in this study were denoted as "hospital A" and "hospital B" to keep the anonymity of hospitals. Also, patients' clinical information was not taken for this study. Ethical approval for this study was obtained from the ethical review committee of the National Hospital of Sri Lanka. Also, administrative approvals were obtained from the relevant authorities at each hospital before conducting the study. The lumbar spine AP and LAT examinations acquired in the supine position were considered for this study. The manual exposure control mode (MEC) was used in both hospitals to perform X-ray examinations. The dosimetric data were only taken from radiographic examinations that contained adequate diagnostic information. Patient-specific parameters such as sex, age, and weight were obtained from each patient. Also, radiographic exposure parameters (tube voltage (kVp) and tube current-exposure time product (mAs)) applied for individual examinations were recorded, and the resulting KAP values were measured using a calibrated KAP meter (VacuDAP Bluetooth, manufactured by VacuTec GmbH, Germany). The data obtained were statistically analyzed using IBM SPSS Version 26.0, and boxplots were utilized to illustrate the variability of patient doses. The normality of the data distribution was assessed by Shapiro–Wilk test at a 95% confidence interval.

III. RESULTS AND DISCUSSION

The study group consisted of 164 adult patients (47.6% males and 52.4% females) who were referred to the radiology departments of the two public hospitals in Sri Lanka. Descriptive statistics of patient-specific parameters (age, sex, and weight) are summarized in Table 1. The mean (range) of the age and weight of the study patient groups were found to be 53.6 (18–84) years and 57.8 (34.2–87.6) kg, respectively. The mean patient weight found this study was close to the mean weight of the adult patients in Sri Lanka, which was 58 kg [8]. As shown in Table 1, the mean weights were approximately similar across patient groups. However, the mean patient dose values were greatly varied but normally distributed for lumbar spine AP and LAT examinations in two hospitals. Conversely, the kVp and mAs values showed a non-normal distribution.

Table 1. Descriptive statistics of patient-specific parameters in two hospitals.

Hospital	Hospital A		Hospital B	
Projection	Lumbar Spine AP	Lumbar Spine LAT	Lumbar Spine AP	Lumbar Spine LAT
No. of Exposures	44	44	38	38
Males	17	17	22	22
Females	27	27	16	16
Age (Years)	53.0 (18-78)	54.0 (18-84)	53.8 (18-84)	53.7 (18-84)
Weight (kg)	57.1 (36.4-87.6)	57.0 (36.4-87.6)	59.0 (34.2-82.9)	58.3 (34.2-82.9)

*Age: mean (range), Weight: mean (range)

Table 2 demonstrates the distribution of KAP values and exposure parameters (kVp and mAs). The mean KAP value in hospital B (1.75 Gy.cm^2) was 200% higher than in hospital A (0.87 Gy.cm^2) for lumbar spine AP examinations. Moreover, there were significant differences in mean kVp values (Mann-Whitney U-test, $p<0.05$) between hospitals A and B for lumbar spine AP and LAT examinations. Figure 1 depicts the boxplots for lumbar spine AP and LAT examinations in two hospitals to better visualize the variability of patient dose values.

Table 2. Descriptive statistics of the KAP and exposure parameters in two hospitals

Hospital	Hospital A		Hospital B	
Projection	Lumbar Spine AP	Lumbar Spine LAT	Lumbar Spine AP	Lumbar Spine LAT
KAP (Gy.cm^2)	0.87 (0.29-1.64)	2.00 (0.73-3.90)	1.75 (0.61-3.03)	1.74 (0.84-2.49)
kVp	67.8 (58-75)	73.6 (68-80)	71.9 (63-84)	78.8 (71-88)
mAs	34.2 (18.0-56.0)	60.2 (28.0-80.0)	35.1 (28.0-40.0)	35.7 (25.0-50.0)

*KAP: median (range), kVp: mean (range), mAs: mean (range)

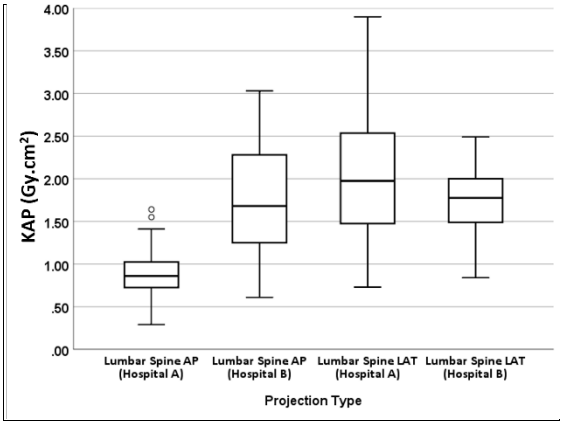

Figure 4. Variations in patient doses in two hospitals for lumbar spine AP and LAT examinations.

The patient doses (KAP values) obtained for the lumbar spine AP and LAT examinations showed a wider dispersion and variability, evidenced by a larger range and high standard deviation (SD) values: 0.29-1.64 (SD = 0.29) Gy.cm^2 and 0.61-3.03 (SD = 0.59) Gy.cm^2 for lumbar spine AP (hospital A and B) and 0.73-3.90 (SD = 0.77) Gy.cm^2 and 0.84-2.49 (SD = 0.41) Gy.cm^2 (hospitals A and B). These dose variations could be mainly due to the manually set exposure parameters (kVp and mAs) and collimation adjustments for patients with different body sizes in two hospitals (proper collimation is important since the irradiated area is proportional to the KAP). Generally, patients with larger body sizes require relatively higher exposure parameters to obtain radiographs. Accordingly, wide ranges of kVp and mAs values were found in two hospitals. For hospitals A and B, the kVp ranges were 58-75 and 63-84 for lumbar spine AP examinations and 68-

80 and 71-88 for lumbar spine LAT examinations, respectively. Likewise, the mAs ranges were 18.0-56.0 and 28.0-40.0 for lumbar spine AP examinations and 28.0-80.0 and 25.0-50.0 for lumbar spine LAT examinations, respectively.

As shown in Table 2, the mean mAs value in hospital A (60.2) was considerably higher than in hospital B (35.7) for lumbar spine LAT examinations, which resulted in higher KAP values. In addition, the resulting KAP values may also be influenced by the radiography systems used and their conditions; however, the same modalities (SHIMADZU DR systems) were used in two hospitals. The extensive variability of patient doses for lumbar spine AP and LAT examinations found in the two hospitals clearly indicated that further radiation dose reduction is possible without compromising the clinical outcome of the examinations. Therefore, the radiology staff should be continuously aware of patient dose levels in order to select the most appropriate radiographic techniques. The findings of this study will also be useful for optimizing individual radiology practices in hospitals associated with large patient dose variations in Sri Lanka.

IV. CONCLUSIONS AND RECOMMENDATIONS

Patient dose monitoring is important to ensure the quality of radiology practices at an optimum level. The excessive variability of radiation doses delivered to patients for the same examination in this study emphasizes the need to introduce reference dose levels for X-ray examinations performed in hospitals in order to optimize patient doses. Furthermore, periodic dose assessments are recommended for hospitals to investigate patient dose variations for all X-ray examination types (especially for X-ray examinations with higher radiation doses) performed in hospitals across the country.

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FOCUS AREA

Energy

SYNTHESIS OF EXPANDED VEIN GRAPHITE VIA ANODIC EXFOLIATION USING SULFURIC ACID AS AN ELECTROLYTE

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Abstract: The electrochemical exfoliation of graphite has been recognized as a cost-effective eco-friendly method that consumes lower energy compared to mechanical, chemical, and thermal exfoliation. However, detailed electrochemical exfoliation of vein graphite which possesses higher crystallinity and purity is limited. Therefore, this study aimed to find out the possibility of synthesizing expanded graphite using vein graphite as a raw material via electrochemical exfoliation. The anodic exfoliation was carried out with the vein graphite rod and Pt rod as the working and reference electrodes, respectively with H_2SO_4 as an electrolyte under 1V for 30 minutes. Crystallographic and morphological analyses revealed an excessive volume increase in graphite layers caused by the electrochemical exfoliation process. Raman spectroscopy analysis showed that the oxidation of the graphite via electrochemical exfoliation had increased the defect density of the edge plane and basal plane in graphite layers. Further, FTIR analysis showed that the distinct peaks appeared on electrochemically exfoliated graphite indicating that oxidation occurred during the anodic exfoliation process. Therefore, it suggests that this produced exfoliated graphite has a highly oxidized and defective nature. Overall, this study demonstrates the possibility of producing expanded vein graphite using the straightforward and affordable electrochemical exfoliation method using H_2SO_4 as the electrolyte.

Keywords: Expanded graphite, electrochemical exfoliation, sulfuric acid

I. INTRODUCTION

The significant characteristics of graphite-based materials, including their high thermal, electrical, mechanical, and permeability qualities, have made them one of the most researched materials in human history [1]. The majority of graphite-based materials are also used in a variety of electronic applications, including solar cells, biosensors, rechargeable batteries, aeronautics, and energy storage devices [2].

Naturally occurring graphite (NG), on the other hand, occurs as a non-functional material due to its hexagonal layered structure. Amorphous graphite, flake graphite, and vein graphite are the three types of NG. The only country in the world that can produce high-purity (about 95-99 % of pure carbon), crystalline vein graphite on a commercially viable basis is Sri Lanka [3].

However, NG is not directly used for advanced applications. As a result, researchers have focused their efforts on modifying natural graphite through mechanical, thermal, or chemical structural modification methods [4]–[7].

Vein graphite is used to produce expanded graphite and improve its quality by using structural modification techniques. Previous studies demonstrated that the use of microwave irradiation technique can produce expanded graphite and it required higher energy for the exfoliation process [7]. Also, chemical intercalation using HNO_3, and exfoliated graphite preparation required a longer time to exfoliate process [6].

The electrochemical exfoliation technique has been recognized as a promising way for structurally modifying the graphite to produce expanded graphite among the numerous methods [8]. Since it can be performed at room temperature in less time and with greater efficiency, it is a more cost-effective and environmentally friendly method that uses less energy than mechanical, chemical, and thermal exfoliation methods [4].

Previous studies demonstrated that the electrochemical exfoliation of highly ordered pyrolytic graphite with 0.5 mol dm^{-3} H_2SO_4 as electrolyte under +3 V potential varied the duration of 5 s to 600 s can be produced exfoliated graphene oxide by a two-stage process [9]. Also, high-quality graphene flakes were prepared using graphite nanoplatelets with 0.1 mol dm^{-3} H_2SO_4 by supplying 10 V potential for 180 s [10]. These studies used flake and synthetic graphite, and higher voltages. There has been no research conducted to study the feasibility of employing electrochemical exfoliation to the natural vein graphite using sulfuric acid as an electrolyte, as well as lower voltages using sulfuric acid. Furthermore, limited investigations on electrochemical exfoliation of vein graphite carried out so far [11], [12].

Therefore, this research aimed to find out the feasibility of producing expanded graphite from Sri Lankan vein graphite via anodic electrochemical exfoliation with H_2SO_4 as an electrolyte.

II. METHODOLOGY AND EXPERIMENTAL DESIGN

A graphite rod (diameter 1 cm x length 10 cm) cut from vein graphite was obtained from the Kahatagaha graphite mine. Direct Current (DC) voltage supplier (EDU-LAB

power supply; 605-056) was obtained from Uva Wellassa University of Sri Lanka. Sulfuric acid (H_2SO_4) was purchased from Sigma Aldrich.

The anodic exfoliation was carried out with the vein graphite rod as the working electrode and electrochemically treated for 30 minutes under 1V DC voltage using a Pt rod as a reference electrode with 500 ml of 1 mol dm^{-3} H_2SO_4 as an electrolyte. The exfoliated material in the solution was filtered and washed with distilled water until the solution pH become neutral (pH=7). This was assisted with vigorous stirring and vacuum filtering. When it became neutral, the sample was oven dried at 120 °C for 1 hour.

Natural vein graphite (RAW) and synthesized expanded graphite (EG) materials were characterized using the X-ray Diffractometer (XRD), Raman spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Particle Size Analyzer (PSA) and Scanning Electron Microscopy (SEM).

III. RESULTS AND DISCUSSION

Figure 1 shows the X-ray diffractograms of raw vein graphite (RAW) and the electrochemically treated expanded graphite (EG) sample for crystalline phase analysis and the calculated interlayer distance (d-space) of the materials. Figure 1 shows the characteristic Gr (002) peak and Gr (004) peak in the RAW sample, indicating the high crystallinity and high purity. The intensity of the 002 peak decreases significantly in the electrochemically treated EG sample than in the RAW sample. Moreover, Figure 1 shows that the primary ($d_{(002)}$) peak has broadened, affecting the highly crystalline nature of this natural vein graphite after the electrochemical exfoliation. The interlayer distance was calculated related to the prominent peak ($d_{(002)}$) using Bragg's law [13] and it revealed that the interlayer spacing of vein graphite had increased from 0.33859 nm to 0.33986 nm after the electrochemical exfoliation process. Based on the XRD results, the crystalline structure of the natural vein graphite may have been subjected to exfoliation and expansion due to the anodic electrochemical exfoliation.

After the anodic electrochemical exfoliation processes, powder RAW and

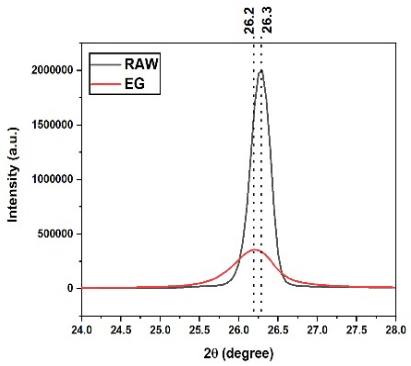

Figure 5. X-Ray diffractograms obtained for RAW and EG sample

electrochemically treated EG samples were characterized by Raman spectroscopy to explore the structural and chemical changes (Figure 2). The significant D peak at ~1350 cm^{-1} which results from the breathing mode of the sp^2 carbon atoms is considered to be a sign of disorders due to edges, functional groups such as hydroxyl and epoxide groups, and the band is activated by the presence of the defects [14], [15]. The G band at ~1580 cm^{-1} is also a characteristic peak of graphite and increases due to in-plane stretching of sp^2 hybridized C atoms

[16]. The ratio of the intensity of the D peak and G peak (I_D/I_G) of Raman spectroscopy was used to estimate the average defect density on the graphite structure after the electrochemical exfoliation (Figure 2) [9]. The I_D/I_G ratio is related to structural defects and is known as a quality indicator of the produced material, which is having high value of the ratio related to the small-sized crystals or increased defect density [17]. The ratio of I_D/I_G of the edge plane of the graphite increased from 0.48 of RAW to 0.7 of EG after the anodic exfoliation. Similarly, the I_D/I_G of the basal plane of the graphite increased from 0.17 RAW to 0.46 of EG. This reveals that the average defect density on the graphite edge and basal surface increased after the electrochemical exfoliation (Figure 2). During the exfoliation process, the I_D/I_G value was increased due to the oxygen functional groups, which causes disorder at carbon edges [18]. According to Xia et. al 2020, the I_D/I_G value for the exfoliated graphite from highly ordered pyrolytic graphite was given 0.81 for 60 s, indicating that the cracks due to blistering were exposing fresh, less defective layers of the underlying bulk graphite. Hence, the present study shows that fewer defective layers with cracks. These observations are consistent with earlier research [19].

The number of graphene layers is determined using the 2D band of the Raman spectrum. However, the 2D band is too weak to determine the number of layers in exfoliated graphite because the stacking order of adjacent layers ruptured during electrochemical oxidation, vibrational signals from the edges of graphite, and introduced amorphized carbon atoms [9], [10]. The behavior of the Raman spectrum shows that the exfoliated graphite possesses a high defect density with a crystalline structure [19].

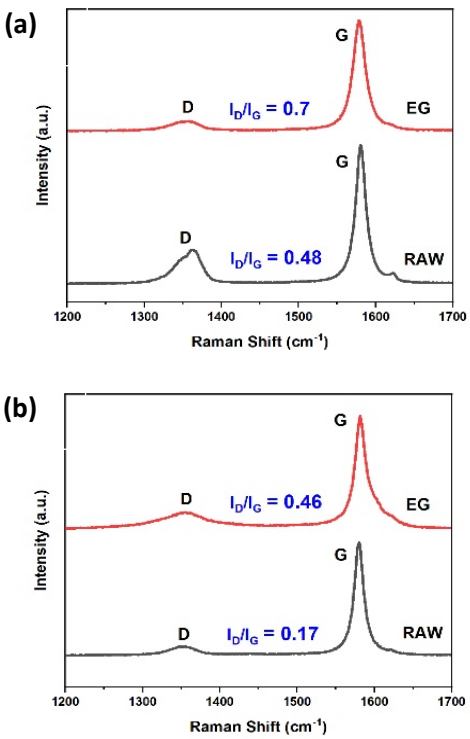

Figure 6. Raman spectra obtained for Edge (a) and Basal (b) planes of RAW and EG sample

The FT-IR spectrum of the RAW and electrochemically treated EG sample is shown in Figure 3. According to Figure 3, in the spectrum of RAW, there are no significant peaks relevant to any functional groups, however, there are noticeable differences in the EG spectrum with a number of distinct peaks appearing in the spectrum. The strong broad vibrational bands at 3200 cm^{-1} - 3600 cm^{-1} is responsible for stretching vibrations of the hydroxyl group, where the hydroxyl groups may be from absorbed water molecules or phenolic

OH^- or OH^- from carboxylic groups [20]. The doublet band at ~2356 cm^{-1} corresponds to absorbed CO_2 molecules [20], and the sp^2 hybridization of the aromatic C=C bond was confirmed by a peak at ~1580 cm^{-1} in the EG sample. EG sample has shown deformation vibration of C-OH, which can be identified at 1350 cm^{-1} [21]. There isn't any information about sulfate-containing groups in spectra. It indicates that vacuum filtration and washing procedure have successfully removed the anions from the sample.

The presence of OH bonds in the EG sample may responsible for the oxidation of natural vein graphite (RAW) via the electrochemical exfoliation process (Figure 3). Moreover, the oxidation may be the reason for an increment of the interlayer distance of graphene layers and an increment of I_D/I_G ratio along with defect density and structural disorder in RAW graphite structure [19].

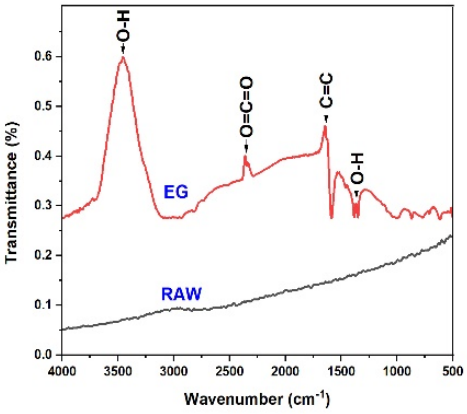

Figure 3. Fourier Transform Infrared Spectroscopy (FTIR) spectra obtained for RAW and EG sample

Figure 4 shows the scanning electron microscopic (SEM) images of RAW and EG samples. Natural vein graphite has thin platy appearance with ideal layer structure characteristics to highly crystalline graphite (Figure 4. a). SEM image of EG (Figure 4. b) revealed an ideal layer-by-layer structure with increased interlayer spacing. Further, an analogous long-range-ordered layered microstructure composed of many translucent and wrinkled paper-like graphene sheets with fluffy morphology of edges can be clearly identified in the SEM of EG (Figure 4. b).

Particle size analysis of expanded graphite was calculated by using the laser diffraction method. A median particle size of 1260.6 nm and a polydispersity index value of 0.878, indicating the broad size distribution of particles, were reported for the EG sample (Figure 5).

In summary, the possibility of synthesizing expanded graphite using Sri Lankan vein graphite as a raw material via the electrochemical exfoliation method has been investigated. Crystallographic and morphological analyses revealed an excessive volume increase in graphite layers caused by the electrochemical exfoliation process.

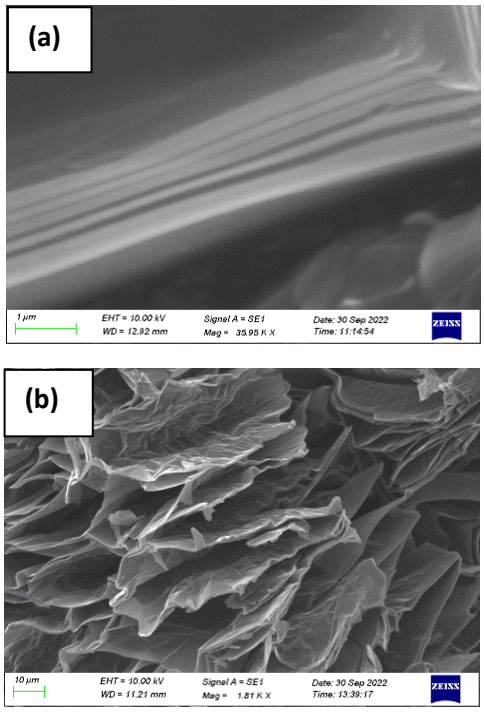

Figure 4. Scanning electron microscopic images obtained for RAW (a) and EG (b) sample

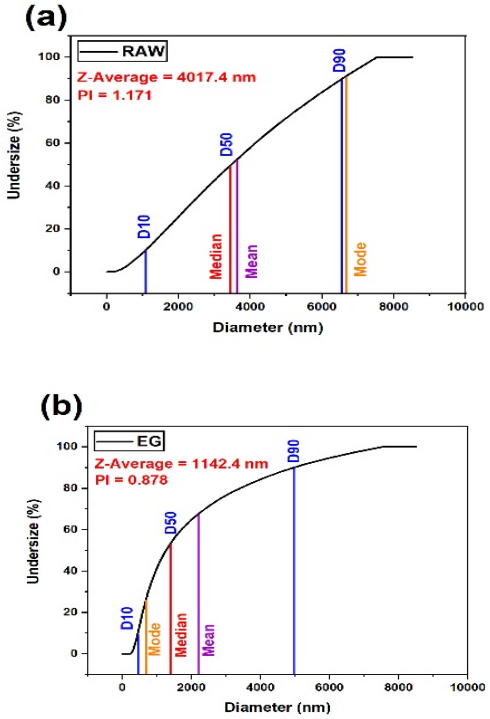

Figure 5. Particle size distribution graphs of RAW (a) and EG sample (b)

Raman spectroscopic results revealed that the defect density of the edge plane and basal plane in graphite layers had increased due to the oxidation of the graphite via electrochemical exfoliation. FTIR and SEM analysis revealed that the oxidation occurred during the anodic electrochemical exfoliation process and showed the defective nature of expanded graphite. Therefore, it suggests that this produced exfoliated graphite has a highly oxidized and defective nature. Furthermore, this study shows that the expanded graphite has a higher crystallinity with fewer defects when compared with previous research [9], [10].

IV. CONCLUSION AND RECOMMENDATION

Overall, this study demonstrates the possibility of producing expanded vein graphite using the straightforward and affordable electrochemical exfoliation method using H_2SO_4 as the electrolyte. Additionally, the electrochemical exfoliation process makes it challenging to generate single graphene EG layers due to the inadequate intercalation of HSO_4^- anions in acid. Material characterization and optimization of parameters such as electrolyte concentration and DC voltage, are currently undergoing to obtain expanded graphite for the investigations in intended rechargeable battery applications and obtained single-layer graphene.

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