

PROCEEDINGS OF THE 10TH YSF SYMPOSIUM

MARCH 18, 2022

Young Scientists Forum

National Science and Technology Commission



10TH YSF SYMPOSIUM

18th March 2022



Organized by

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National Science and Technology Commission

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Message from the Director National Science and Technology Commission

I am pleased to convey this message to the 10th YSF Annual Research Symposium of the National Science and Technology Commission (NASTEC) organized by the Young Scientists Forum (YSF) of NASTEC.

The YSF Symposium which was initiated by the NASTEC in 2012 has now reached its 10th year amidst several challenges. Especially the activities of YSF were hampered severely over the past two years as a result of COVID 19. However, I am indeed grateful for the Steering Committee and the members of the YSF for their tireless effort in continuing the activities of YSF.

As in the past, this year's symposium is also focused on promoting Research and Development together with stimulating technology based startups in line with the thrust areas identified in the National Research & Development Framework (NRDF); the National Framework of the country. The thrust areas include Health, Food, Nutrition & Agriculture, Environment, Water, Energy, Shelter, Mineral Resources, Information Communication Technology & Knowledge Services, Basic Sciences, and Emerging Technologies & Indigenous Knowledge. However, the review panel ensured that the research papers selected for the 10th YSF symposium has clear link in addressing country's priority issues of identified sectors. As such, outcome of the 10th YSF Symposium considered more important to NASTEC in providing required scientific advice to the decision makers.

One of the key objectives of the YSF is to provide a platform for the young scientists to share their knowledge and experiences of novel research interventions while mobilizing their collective intellect to improve the performance of Science, Technology and Innovation (STI) sector in the country. Therefore, I trust the young scientists of the YSF forum will provide their continuous contribution in realizing this objective.

I congratulate all the young scientists who present their research papers at the 10th YSF symposium and all other young scientists of the YSF while wishing good luck with the proceeding of the sessions

Mrs. Nazeema Ahamed

Act. Director/CEO

National Science and Technology Commission

Message from the Steering Committee Chairperson Young Scientist Forum

I am delighted and honored to bring this message for the 10th Annual YSF Symposium as the Chairperson of the Steering Committee of the Young Scientists Forum. This year the scientists have faced unprecedented challenges due to the COVID 19 global pandemic. However, these young scientists have found ways to uphold their continued enthusiasm for research. Therefore, this symposium will provide valuable opportunities for a productive discourse and aspiring excellence in research.

The purpose of organizing this symposium is to give young scientists an opportunity to address scientific concerns and present their research discoveries in crucial fields such as Food, Nutrition, and Agriculture, Environment, Energy, ICT, Basic Sciences, and Emerging Technology, among others and thereby pave a platform to showcase their innovative research. This symposium is in accordance with NASTEC's goal of involving youth in constructive scientific activities, as well as serving as a forum for young scientists to foster networking and provide a unique chance for collaborative research by discussing current research findings, future plans, and research interests. Young scientists will be exposed to the highest standards of research early on, and they will develop a rigor that will favorably affect their future discoveries, owing to chances like these.

Recognizing the current situation, this symposium has been well structured to address the challenges head on. At this occasion, I convey my sincere gratitude to Mrs. Nazeema Ahamed, the Acting Director of the NASTEC for her tremendous support extended to making the YSF Symposium a success. I thank the steering committee for their wise advice and brilliant suggestions on organizing this symposium. All recognition should go to the steering committee members who have all worked extremely hard on the details of important aspects of the symposium programs. A special note of appreciation goes to the academia for their thorough and timely reviewing of the papers and for maintaining standards of the EA's presented at the symposium. Furthermore, I'd like to express my gratitude to the Editorial Board and Chief Editor for their tireless efforts in compiling the symposium proceedings. I also thank the presenters, for enriching this symposium by your presence and all the authors for their outstanding contributions. A special appreciation goes to Ms. Thilini Munagamage, Scientist of NASTEC and the symposium coordinator, for her efforts on all the YSF activities.

I wish the 10th Symposium of YSF a grand success.

Dr Sameera R Samarakoon
Chairperson - YSF Steering Committee

Foreword from the Editors

It is with great pleasure, the Young Scientist Forum (YSF) present the proceedings of the 10th 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 57 extended abstracts and 23 full papers received for the year 2022, 39 extended abstracts and 17 full papers 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 Mrs. Nazeema Ahamed, the 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 10th YSF symposium a great success and extend warm wishes to all the authors.

The Editorial Board

10th YSF Symposium Proceedings

EXTENDED ABSTRACTS

FOCUS AREA

**Basic Sciences, Emerging Technologies &
Indigenous Knowledge**

REVIEW ON THE EFFECTIVENESS OF ARKAMULADI LEPA IN THE MANAGEMENT OF RHEUMATOID ARTHRITIS

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Introduction

Rheumatoid Arthritis (RA) is an autoimmune chronic inflammatory disorder. The characteristic feature of RA is persistent inflammatory synovitis that usually involves in peripheral joints in a symmetric distribution, where synovium becomes inflamed causing warmth, redness, swelling and pain. As the disease progresses, the inflamed synovium invades and damages the cartilage and bone of the joint. The potential of the synovial inflammation to cause cartilage destruction is the hallmark of the disease [1]. RA is a chronic disease affecting over 350 million populations worldwide [2].

According to Ayurveda, RA is similar to *Amavata*. *Amavata*, as a disease, was first described in detail by Madhavakara in Madhava Nidana characterized by pain, stiffness and swelling over joints [3].

Presently, non-steroidal anti-inflammatory drugs (NSAIDs) are the main stay in Allopathic medicine for this condition. However, they show adverse effects, and have limitations in long term therapies. The immunosuppressive drugs are reserved for selected cases, while the disease modifying drugs like gold-salts are costly and have low benefit risk ratio. Hence, there is a need for drugs having good efficacy with no toxicity for this debilitating disorder. A number of indigenous drugs have been claimed to be effective in the treatment of RA but their claims have not been largely substantiated in well-controlled clinical trials.

Herbal remedies exert less side effects compared to Allopathic medicines. A vast number of formulations have been mentioned in “Piyusharnava” for RA related disorders. Majority of those formulations have not studied scientifically to establish the pharmacological properties in the management of RA. Hence, the “*Arkamuladi Lepa*” is selected in this literature study. The formulation of “*Arkamuladi Lepa*” consists of *Caltropis gigantea* (sans; Arka), *Zingiber officinale* (sans; Shunti), *Acorus calamus* (sans; Vacha) and *Brassica nigra* (sans; Sarshapa) and the grinding liquid is cow’s urine [4].

The general objective of this study was to review on the pharmacological properties of *Arkamuladi Lepa* in the management of RA and the specific objectives were to identify the taste (*Rasa*), quality (*Guna*), potency (*Virya*),

metabolism (*Vipaka*), eradication of vitiated humors (*Doshaghnata*), specific pharmacological actions (*Karma*), modern pharmacological actions and chemical composition of the ingredients of *Arkamuladi Lepa* in the management of RA.

Materials and Methods

Identification of the ingredients that are used for the preparation of *Arkamuladi Lepa* mentioned in Piyusharnava and the *Pancha padartha* (*Rasa, Guna, Virya, Vipaka and Prabhava*), *Doshaghnata, Karma* of ingredients according to the relevant Ayurveda Materia Medica. Also the other objective is identification of the Pharmacology, Pharmacognosy, Chemical composition, Anti- inflammatory and Analgesic activities of the ingredients through modern botanical texts and previous research papers. Finally, analyzed the collateral data which were obtained, by using charts and graphs.

Table 1. Analysis of ingredients based on five elements, eradication of vitiated humors, specific pharmacological actions, modern pharmacological actions and chemical composition

Ingredient Property	<i>Caltropis gigantea</i>	<i>Zingiber officinale</i>	<i>Acorus calamus</i>	<i>Brassica nigra</i>	<i>Cow's urine</i>
Taste	<i>Katu, Tikta</i>	<i>Katu</i>	<i>Katu, Tikta</i>	<i>Katu, Tikta</i>	<i>Tikta, Kashaya</i>
Quality	<i>Laghu</i>	<i>Laghu, Snigdha</i>	<i>Laghu, Tikshna</i>	<i>Laghu, Snigdha</i>	<i>Tikshna</i>
Potency	<i>Ushna</i>	<i>Ushna</i>	<i>Ushna</i>	<i>Ushna</i>	<i>Ushna</i>
Metabolism	<i>Katu</i>	Madhura	<i>Katu</i>	<i>Katu</i>	<i>Katu</i>
Eradication of vitiated Humors	<i>Kaphavata hara</i>	<i>Vatakapha hara</i>	<i>Kaphavata shamaka</i>	<i>Kaphavata shamaka</i>	<i>Kapha hara vata hara</i>
Specific pharmacological actions	<i>Shothahara, Vedanasthapana</i>				
Modern pharmacological actions	<i>Anti-inflammatory, Analgesic</i>				
Chemical composition	<i>Flavanoid, Alkaloid, Saponins, Tanin, Steroid, Glycosides</i>				<i>Phenols, Protein & Amino acids</i>
	<i>Carbohydrate s, Protein & Amino acids</i>	<i>Terpenes, Phenols, Carbohydrates, Protein & Amino acids</i>	<i>Phenols, Triterpenoids, Carbohydrates</i>	<i>Terpenes, Phenols, Polyphenoles</i>	

Results and Discussion

According to Ayurveda, *Amavata* occurs due to the vitiation of *kapha dosha* and *vata dosha*. Collected data reveals that most of the drugs have equal properties. The ingredients of the formula predominantly (80%) possess *Katu rasa* as well as *Tikta rasa* and followed by *Kashaya rasa* (20%). Based on *Guna* it possesses *Laghu* (80%), *Snigdha* (40%) and *Tikshna guna* (40%). As well as the ingredients possess *Ushna virya* (100%). The majority of ingredients having *Katu vipaka* (80%) while remaining ingredients having *Madhura vipaka* (20%). All the ingredients possess *Kapha hara* (100%) action and *Vatahara* (100%) action.

All the ingredients possess *Kapha hara* and *Vata hara dosha karma*. Hence, *Katu-Tikta- Kashaya rasa*, *Laghu-Teekshna guna*, *Ushna virya* and *Katu Vipaka* responsible for the *Vata* pacification while *Snigdha guna*, *Ushna virya* and *Madhura vipaka* responsible for the *Kapha* pacification.

When considering about Ayurvedic pharmacological actions (*Karma*) it is having *Shothahara* (100%), *Vedanasthapana* (100%) *Karma*. *Vedanasthapana* action occurs due to the presence of *Tikta*, *Kashaya*, *Katu Rasa*, *Snigdha*, *Ushna guna* and *Ushna virya*. Hence herbs related to *Vedanasthapana gana* having the ability to reduce the swelling as well as pain. They are utmost useful in joint pain and muscle pain as they are act as an Analgesic and an Anti-inflammatory agent.

According to the modern view, it is efficient in the management of RA because of its mechanism including Anti-inflammatory (100%), Analgesic (100%), Anti-oxidant (100%) and Immunomodulatory (60%) actions. Majority of the ingredients of formula possess Anti-inflammatory and Analgesic actions.

Present study revealed that the majority of chemical composition of *Arkamuladi Lepa* possess Anti-inflammatory, Analgesic and Anti-oxidant effects. According to the results, most of the ingredients possess (80%) Flavanoids, Alkaloids, Saponins, Phenols, Tanins, Steroids and Glycosides, while (60%) possess Carbohydrates, Protein and Amino acids, followed by (40%) possess Terpenes and (20%) possess Triterpenoids and Polyphenols.

Based on research papers, majority of chemical classes found in the formula possess Anti-inflammatory and Analgesic actions. Alkaloids, Steroids, Flavanoids, Tanins and Carbohydrates possess moderate Analgesic activity as well as Natural Flavanoids, Steroids and Triterpenes Posses Anti-inflammatory actions. Polyphenols, Proteins, Terpenes, Phenols, Glycosides and Triterpenes also have been detected as potent Anti-arthritis phytochemicals with Anti-inflammatory and Analgesic activities. Due to the presence of these chemical constituents, the formula which was analyzed in this study can be proved as effective in the management of RA.

Conclusions and Recommendations

Based on the analyzed evidences, the formula of *Arkamuladi Lepa* mentioned in Piyusharnava, can be effectively used to manage RA based on its pharmacological properties as an external application.

I recommend this study to continue as a clinical study to evaluate the effect and efficacy of *Arkamuladi Lepa* in the management of RA.

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SYNTHESIS OF A SURFACE MODIFIED NANO SILICA FILLER FOR NATURAL RUBBER COMPOSITE USING RICE HUSK ASH

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Introduction

Sri Lanka's paddy production is around 4.82 million tons per year, of which 20% contributes to the production of rice husks (RH), which is an environmentally harmful by-product. RH needs a large area for disposal and take a long time to degrade completely, and greenhouse gases are emitted during decomposition. There is a high risk of fire in these areas as well. Rice husk is used as an energy source as it has a high calorific value. The ash content of RH is 20% wt, of which 98% SiO₂ is present. Therefore, rice husk ash (RHA) is an environmentally friendly and ecologically friendly silica source.

Silica is one of the popular and effective reinforcing fillers used in natural rubber (NR), which is the most popular elastomer or flexible hydrocarbon polymer produced by nature. Modern fuel-efficient tires use silica because of its lower rolling resistance and better wet grip than other fillers. Silica shows poor dispersion in the NR matrix due to strong filler-filler interaction of silica. Silanol and siloxane groups are added to the silica surface, that form a polar and hydrophilic surface of silica and lead to strong particle-particle interactions through hydrogen bonds. However, this causes long cure times because the silane groups in silica are acidic and retain accelerators and reduce crosslink density.

Particle size, structure, specific surface area and surface chemistry of the filler particles affect the properties of the composite. In the last two decades, interest in nanofillers has grown rapidly. In micro or convection fillers, aggregates exist in the NR, while nanofillers interact with the polymer. For this reason, nanofiller reinforcing rubber provides high hardness, modulus, anti-aging properties and low swelling. Nanosilica-reinforced composite NR shows good improvement in physical properties in many researches.

The objective of the present work was to add high value to the RHA by producing an effective nanofiller for rubber products. In our study, we prepared NS from RHA as a value addition for RHA. Nanosilica (NS) obtained from RHA was modified

by using Si-69 coupling agent to prepare reinforcing filler for the NR composite to overcome poor dispersion and high curing time.

Materials and Methods

Rice husk was collected from paddy fields around Kandy, Sri Lanka. Analytical grade HCl and Sodium hydroxide (NaOH) were used, (3-triethoxysilylpropyl) tetrasulphone (Si-69)

Nanosilica preparation from RHA

First, the collected rice husks 5 kg were cleaned with distilled water 3 times to remove impurities of solid and dust, and then they were dried at 80 °C for 24 hours. Afterwards, the rice husk was heated in a 2M HCl solution at 80 °C for 3 hours. Subsequently, the HCl was removed, and the rice husk was washed with distilled water three times and dried at 80 °C for 24 hours. After that, dry rice husks were burned in a muffle furnace at 700 °C for 3 hours [1].

Next, 10 g of rice husk ash was dispersed in 160 mL of 2.5M NaOH solution and stirred using a magnetic stir at 80 °C for 1 hour. After that, the sample was filtered and the residue was washed with 40 mL distilled water and collected the filtrate. Then the filtrate was titrated with a 2M HCl solution while string. When the pH reached 2, the titration was stopped and the gel was allowed to age for 24 hours. Then, the gel obtained was fragmented, centrifuged, and washed with distilled water for three times and with ethanol two times. Then the centrifuged-silica was dried at 80 °C for 8 hours.

Nanosilica modification

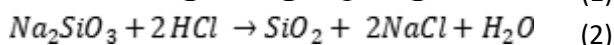
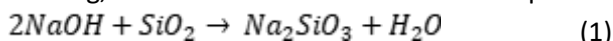
Surface modified nanosilica was prepared by mixing nanosilica from RHA with 8% of Si-69 from silica by mass. Then distilled water was added and mixed well until it became a paste. Then the mixture was dried at 140 °C for 1 hour [2].

Characterize of a modified nanosilica

The particle size of the synthesized silica was characterized with SEM analysis. FTIR used to identify the functional group.

Results and Discussion

When silica in RHA is reacted with NaOH, Na₂SiO₃ is formed, and when adding HCl under vigorous stirring, nanosilica is formed as shown in equation 1,2.



SEM images in figure 1 demonstrate the existence of spherical shaped particles. Spherical particles were in the size of 60-80 nm.

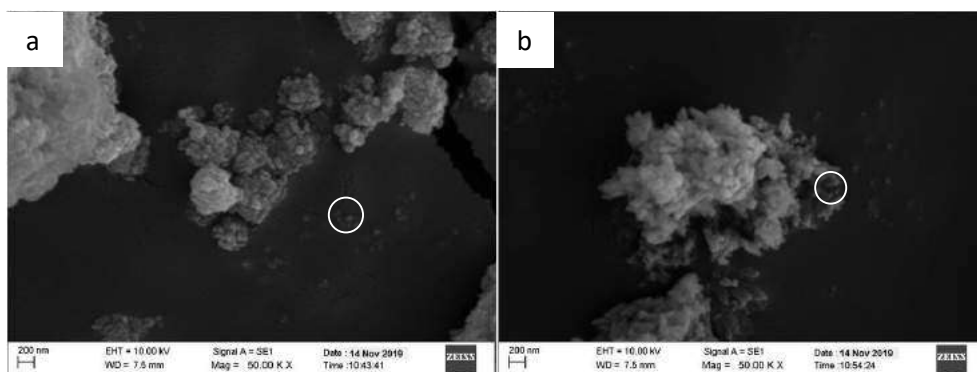


Figure 1. SEM images of synthesized nanosilica particles from RHA, a) SEM obtained from NS Sample 1 and SEM obtained from NS Sample 4

FTIR of nanosilica and modified nanosilica is shown in figure 2. The broad O–H stretching band of nanosilica and modified nanosilica have been observed at 3356 cm^{-1} and 3387 cm^{-1} respectively, in figure 2 [3,4].

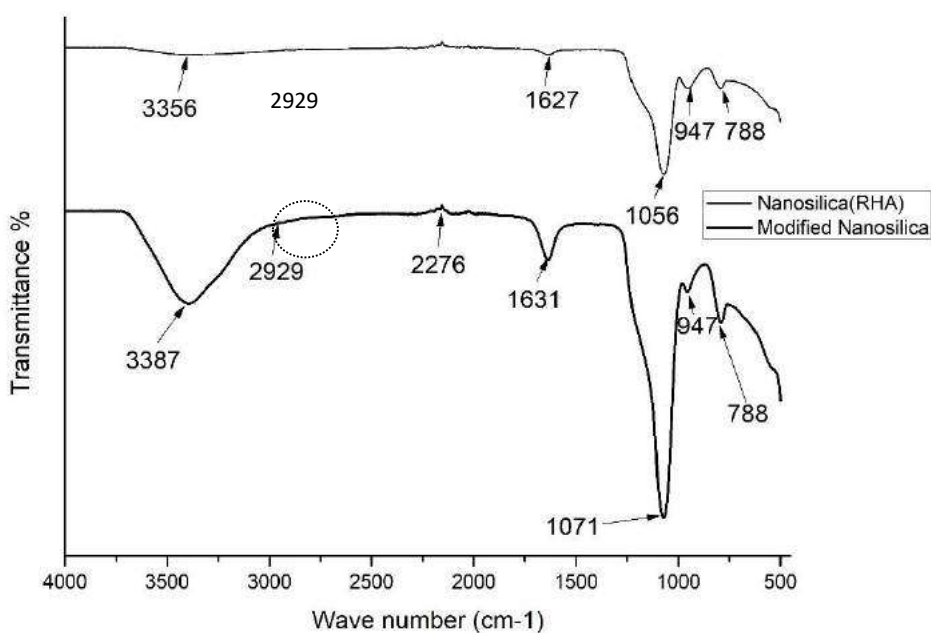


Figure 2. FTIR spectra of synthesized nanosilica and surface modified nanosilica

The asymmetry stretching of Si–O–Si bonds in both linear and cyclic forms of nanosilica and modified nanosilica is observed at 1056 cm^{-1} and 1071 cm^{-1} respectively [4]. Modified nanosilica shows a new peak at 2929 cm^{-1} in figure 3 that is attributed to C–H stretching of CH_2 . In treated nanosilica, the characteristic

peak for C–O appears at 1631 cm⁻¹. These results confirm that the silica surface is modified by Si-69 [3]

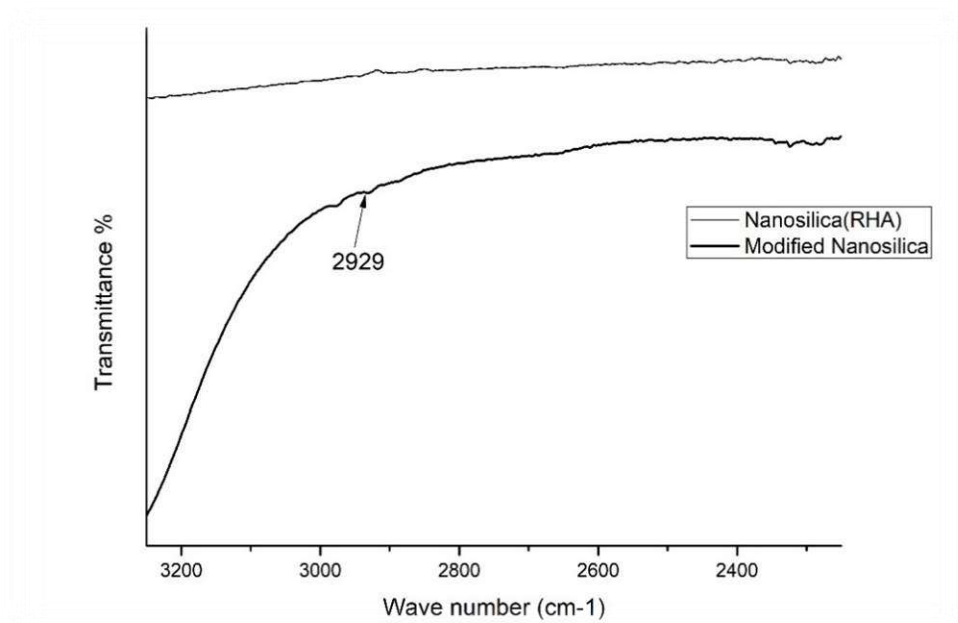


Figure 3. FTIR spectra of synthesize nanosilica and surface modified nanosilica in 3200cm⁻¹ to 2400 cm⁻¹ wave number range

Synthesis of the modified nanosilica using Si-69 silane coupling agent involves the hydrolysis and condensation steps, as shown in figure 4.

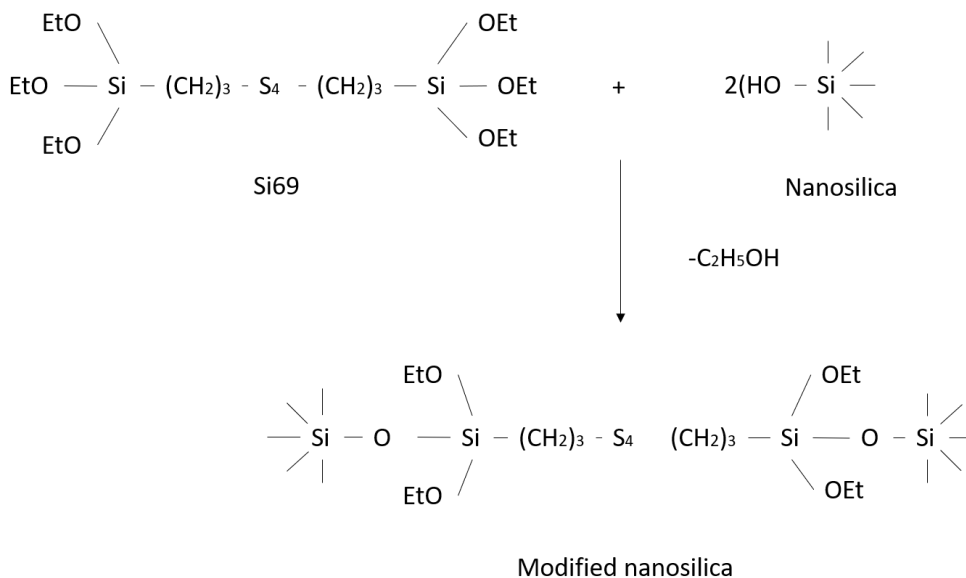


Figure 4. Nanosilica surface modification

The modified nanosilica structure can react with dry rubber under the vulcanization step and contribute to the cross-link formation, which will improve the dispersion of NS into the rubber matrix and will improve the mechanical properties and least liquid sorption. In addition to that, as Si-69 has bonded with NS before being added to the composite, its ability to retain accelerators will be reduced, hence curing time will be reduced [5].

Conclusions and Recommendations

Using the method reported in this study, we succeeded in fabricating a surface modified nanosilica from RHA that is suitable as a reinforcement filler in natural rubber

Acknowledgments

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UV RADIATION SCREENING POTENTIAL OF SUNSCREEN FORMULATIONS PREPARED FROM *Atalantia ceylanica* (Yaki-narang) EXTRACT

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Introduction

The ultraviolet (UV) radiation that is emitted from natural and artificial sources can cause various detrimental effects on human skin. These can be either acute or chronic. The acute effects are erythema, immediate pigment darkening (IPD), and free radical formation. The chronic effects are more disastrous, and they include premature photoaging and photo-carcinogenesis. The primary precaution to avoid these conditions relies on not letting the skin to get exposed to UV radiation from any source. However, in everyday settings, it is very hard to achieve this, especially, to avoid sunlight which is a major source of UV radiation. Therefore, various strategies have been introduced to overcome the detrimental effects caused by UV radiation. This includes approaches such as reducing the amount of UV radiation penetrating the skin. Sunscreens that contain UV filtering organic or inorganic molecules play a major role in this context [1]. After applying on the skin, the sunscreens can filter out UV radiation by reflection, scattering, and absorption [2,3]. These sunscreens are categorized into two groups as herbal sunscreens and synthetic sunscreens. However, synthetic sunscreens have been identified with causing potential side effects on human skin after application. Therefore, sunscreens that have an herbal origin are more prominent these days. The reason is that their side effects are less than synthetic sunscreens and display high UV absorption values in experimental settings. In the quest of finding new herbal-based sunscreen products, the knowledge from Sri Lankan indigenous medicine plays a vital role. Traditional medicine of Sri Lanka uses various herbs as therapeutics to cure various skin diseases. *Atalantia ceylanica* is a native Sri Lankan plant that uses to cure skin conditions and respiratory complaints [1]. Therefore, it is worth trying to develop herbal sunscreen products from this plant, and subsequently, assess their UV attenuating capability.

Materials and Methods

Preparation of crude extract

The leaves of *A. ceylanica* were collected, thoroughly washed three times using running tap water, and dried in the shade for one week. After one week, the dried leaves were ground until a fine powder was obtained using a domestic grinder. The powdered plant material (40.0 g-50.0 g) was subsequently extracted using 300 mL of methanol, and then the extract was evaporated into dryness using a rotary evaporator.

Preparation of creams

Different weight percentages of plant extract (i.e., 25%, 50% and 75%) and aqueous cream base were mixed to produce three herbal sunscreen products.

Determination of UV absorbance

The UV filtering potential of the prepared herbal sunscreens was determined according to the method described by Napagoda *et al.* (2016). Here, a weight of 10 mg of each sunscreen product was dissolved in 10 mL of distilled water to obtain a concentration of 1mg/mL. Then, the UV absorbance of the solution was measured from 260 nm to 400 nm with 5 nm increments using a UV-visible spectrophotometer against distilled water as the blank. Three measurements were made at each point, and mean absorbance values were calculated.

Determination of sun protection factor (SPF) and photostability

The SPF is a numerical value that represents the UV barring capability of a particular sunscreen product. Here, the SPF values of developed sunscreen products are calculated according to the equation developed by Mansur *et al.* (1986) using the UV absorbance values obtained previously [4].

$$SPF_{\text{spectrophotometric}} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) Abs(\lambda)$$

EE(λ) – erythral effect spectrum; I(λ) – solar intensity spectrum; Abs(λ) – absorbance of sunscreen product; CF – correction factor (=10).

For the experiment, a commercial synthetic sunscreen product with a labelled SPF value of 15 was used as the positive control and, the aqueous cream base was used as the negative control.

The prepared samples were exposed to direct sunlight for 21 days, and subsequently, the SPF values were calculated on the 7th, 14th, and 21st days to determine the photostability. The experiment was conducted in triplicate using distilled water as the blank.

Results and Discussion

Table 1 displays the SPF values obtained for the prepared herbal sunscreens over three weeks. According to these values, it is revealed that immediately after preparation, all the formulations display high SPF values. However, while exposed to direct sunlight, their SPF values are timely decreased. On the other hand, the sunscreen formulation that contained 75% of extract always displayed the highest SPF value throughout the experiment (Table 1). Of interest, the aqueous cream base which was used as the negative control did not display any UV absorptive potential. Moreover, the positive control displayed a lower SPF value of 3.94 ± 0.15 than the labelled value (SPF=15) and this agree with the previous findings [1].

Table 1. SPF values of prepared herbal sunscreen products over three weeks period

Extract percentage (%)	SPF values			
	Initial	7 days	14 days	21 days
25	12.97 ± 0.54	11.83 ± 0.22	8.63 ± 0.10	7.55 ± 0.25
50	15.40 ± 0.35	11.94 ± 0.36	8.61 ± 1.19	7.26 ± 0.40
75	19.13 ± 1.25	13.61 ± 0.21	11.26 ± 1.86	9.18 ± 0.54
Positive control	3.94 ± 0.15	3.85 ± 0.06	3.46 ± 0.07	3.46 ± 0.07
Negative control	0	0	0	0

Data represented as mean \pm SD (n=3).

Figure 1 displays the UV absorption profiles of the sunscreen formulations and the positive control. From the chart, it has been noted that the prepared sunscreens can effectively filter out UV radiation by absorption. Especially, the sunscreens are highly active in the lower UV range. However, the sunscreen with 75% of extract is superior to the other two formulations in the absorption of UV radiation. Besides, the positive control shows a very low UV absorption profile than it is declared.

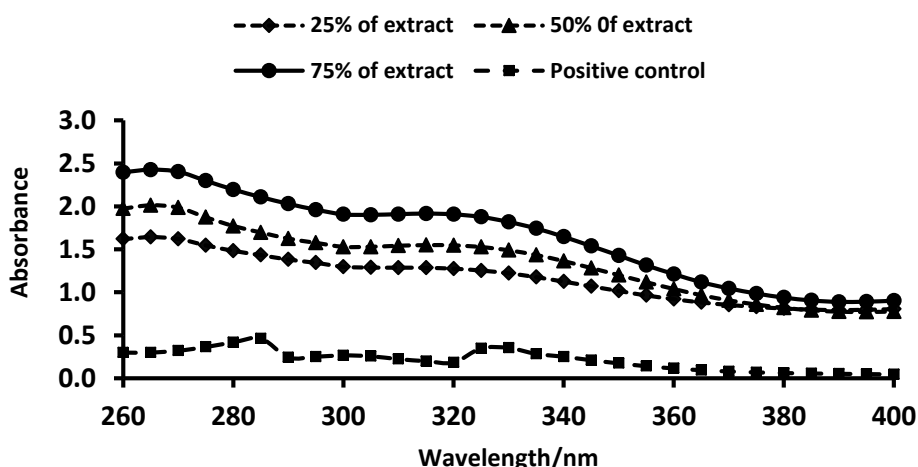


Figure 1. Variation of absorbance throughout the UV range of the prepared sunscreen formulations and positive control

Conclusions and Recommendations

The herbal sunscreens prepared from *A. ceylanica* methanolic extract can be better alternatives to synthetic sunscreen products. However, these sunscreens don't have a good photostability profile that is to keep the sunscreen's activity almost constant throughout a certain period. Therefore, to obtain better protection, these sunscreens must use within one week after preparation. On the other hand, higher plant extract concentration should always be present within the formulation to obtain higher protection. In the meantime, steps must be found to keep the sunscreen's activity stable with time before developing into commercial scale.

Acknowledgement

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AN IN-SILICO STUDY OF POSSIBLE ANTI-INFLAMMATORY ACTIVITY ON IL-1R AND IL-6R USING SELECTED PHYTOCHEMICALS OF PASPANGUWA

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Introduction

Traditional medicines have made impressive progress in treating various diseases. A Large number of herbal plants are known to possess highly therapeutic properties which are useful in treating enormous medical conditions and diseases with minimum side effects. Among them, traditional paspanguwa has taken an immense place in treating conditions such as inflammation. Over the years the recipe of traditional paspanguwa has passed from generation to generation verbally. Other than written in folk notes; “puskola poth” scientific evidence proven for their therapeutic medicinal activities are limited. In the Sri Lankan native language, paspanguwa gives a meaning of five portions, which is a combination of five main herbs. These herbs show higher therapeutic activity against inflammation separately. The five major herbs consisted in paspanguwa are Ginger (*Zingiber officinale*), Corriander (*Coriandrum sativum*), Pathpadagam (*Hedyotis corymbose*), Katuwalbatu (*Solanum xanthocarpum*) and Veniwalgata (*Coscinium fenestratum*).

The main ailments which the paspanguwa provides protection against are cold, cough, fever, headache, and cold-related symptoms. These symptoms are naturally caused by bacterial or viral infections. As the pathogens invade the host, it triggers the production of cytokines. Cytokines are protein mediators which have endogenous pyrogen (EP) like properties. Thus IL-1 and IL-6 are a few cytokines that have proinflammatory properties. IL-1 binds to IL-1R which is responsible for transmitting inflammatory properties of IL-1. IL-6R- α is a glycoprotein that forms a complex with signal transducer protein gp130 to initiate the biological activities of IL-6.

Due to the lack of clinically proven evidences for anti-inflammatory activities of paspanguwa, an in-silico study was carried out to find how the selected phytochemicals of the herbs in paspanguwa that bind to the IL-1R and IL-6R- α there by opposing the effect of IL-1 and IL-6.

Materials and Methods

Plant Review

An extensive literature review was carried out to find the phytochemicals that have anti-inflammatory properties. The 3D structures of phytochemicals obtained from the PUBCHEM [1] were submitted to SWISSADME to predict the drug-likeness and toxicities. Thirty-four phytochemicals were selected based on the Lipinski rule of five.

Selection of crystallographic structure

The crystallographic structures of Interleukin-6 receptor α chain (IL-6R- α) (PDB ID: 1N26) and Interleukin-1 receptor (IL-1R) (PDB ID: 1IRA) [2] were obtained from the RCSB protein database. Modelling was carried out using MODELLER 9.24 [3] and further refined by using REFOLD web server. The 3D models were validated by VERIFY3D, ERRAT, PROCHECK, and PROVE tests offered by the SAVES v5.0 server.

Preparation of molecules and parameters for molecular docking

Protein preparation erstwhile to molecular docking was carried out using AutoDock Tool 1.5.6 [4]. The ligand optimization and energy minimization were done by Avogadro 1.2 using the MMFF94 force field with 500 steps. Binding sites of the proteins were predicted by carrying out a blind docking and confirming the resulted coordinates with other literature[1]. Autogrid 4.2 was used to generate grid maps and grid parameters. The genetic algorithm parameters to prepare dock.dpf file were set as follows: No. of GA runs: 100, population size: 300 and the maximum number evaluations: 250 000 000. Autodock 4.2 was used for rigid docking and creating results.

Results and Discussion

Structure based virtual screening of phytochemicals

Phytochemical screening was performed to select the most suitable phytochemicals to carry out further investigation. Thirty-four phytochemicals were selected based on the Lipinski rule of five and ADMET predictions for Human intestinal absorption and carcinogenicity.

Model validation

The protein structures were modelled using MODELLER 9.24. The compatibility of the 3D structures with its deleted amino acid sequence was done by VERIFY3-D predicting the results as 93.96% and 80.02% for IL-6R- α and IL-1R with 3D-1D

score of ≥ 0.2 . The statistics of nonbonded interactions between different atom types were analyzed using the ERRAT server. Both IL-6R- α and IL-1R had scores of 83.56 and 74.920 respectively. To check the stereochemical quality of the structures Ramachandran plots were examined by PROCHECK. According to the plots, IL-6R- α had 0.8% residues in the disallowed region, while IL-1R had 0.0% residues in the disallowed region. In PROVE test both IL-6R- α and IL-1R had 4.90% and 3.40% respectively. Moreover, the PROSA test was carried out to evaluate the quality of the protein folds of IL-6R- α and IL-1R. They have scored values of -6.31 and -7.79, indicating proper folding.

Molecular docking studies

Various kinds of bacteria and viruses enters into a host, they tend to release toxins, which results in illnesses to the host. Ailments such as cold, fever, nausea and cold related diseases are most common among the society. As mentioned above, paspanguwa is a combination of five main herbs, which were used as a treatment for these ailments. The five main herbs of paspanguwa are namely, Ginger, Coriander, Pathpadagam, Katuwalbatu and Venivalgeta. Here we predict how the phytochemicals of these herbs inhibit the process, by binding to the allosteric sites of the receptors IL-1R and IL-6R- α .

Ginger rhizomes are commonly used as a spice in our daily food. But other than a spice it also has a great anti-inflammatory activity to sicknesses such as vomiting, nausea, morning sickness of pregnant women and etc. Ginger has many active constituents. Phytochemicals that obey the Lipinski rule, was docked to both receptors to predict their binding affinities. Among them, only a few had great binding affinities to both IL-1R and IL-6R- α . 6-Gingerol had binding energies of -5.87 kcal/mol and -5.49 kcal/mol towards IL-1R and IL-6R- α respectively. Zingiberene showed the highest affinity of -6.59 kcal/mol towards IL-1R and -5.32 kcal/mol towards IL-6R- α . Other than that, β -Bisbolene also showed high binding energies towards IL-1R and IL-6R- α as -6.38 kcal/mol and -5.19 kcal/mol respectively.

Coriander is a well-known herb for its anti-inflammatory properties. Among its active constituents, camphor and geraniol showed the highest affinities towards IL-1R (-5.69 kcal/mol and -5.11 kcal/mol respectively) and low towards IL-6R- α (-4.46 kcal/mol and -4.1 kcal/mol). Cineole showed moderately higher affinity towards IL-6R- α as -4.7 kcal/mol and -4.98 kcal/mol for IL-1R, when compared to other docked phytochemicals. Other than that, active compounds such as linalool, limonene, geranyl acetate, α -pinene and γ -terpinene show relatively low binding affinities.

Stem and leaves of Pathpadagam are used in both traditional and modern medicine for their immense anti-inflammatory properties and fever reducing effects. Out of all its active constituents screened and docked, γ -sitosterol showed the highest binding affinity of -10.26 kcal/mol towards IL-1R. Ursolic acid and Oleanolic acid also showed high binding energies towards IL-1R and IL-6R- α (ΔG = -9.15 kcal/mol, -6.69 kcal/mol and -8.87 kcal/mol, -6.29 kcal/mol respectively). Stem and leaves of this are used as one of the five main ingredients in paspanguwa.

Dried berries of Katuwalbatu were used as the ingredient for the paspanguwa. It contains high amounts of alkaloids and flavonoids. The primary bioactive compound of this herb is solasodine. It showed high binding affinities towards IL-1R and IL-6R- α (ΔG = -9.59 kcal/mol and -6.25 kcal/mol). The highest binding energies of *S. xanthocarpum*, were shown by stigmasterol for IL-1R as -10.54 kcal/mol and -6.95 kcal/mol for IL-6R- α . Carpesterol and Diosgenin also showed high binding affinities of -6.77 kcal/mol, -9.7 kcal/mol and -6.28 kcal/mol, -9.28 kcal/mol for IL-6R- α and IL-1R respectively. Linoleic Acid have shown a relatively high binding energy of -6.52 kcal/mol towards IL-1R. Other active compounds such as, apigenin, coumarin, and oleic acid also have shown a moderate amount of binding affinities towards the receptors.

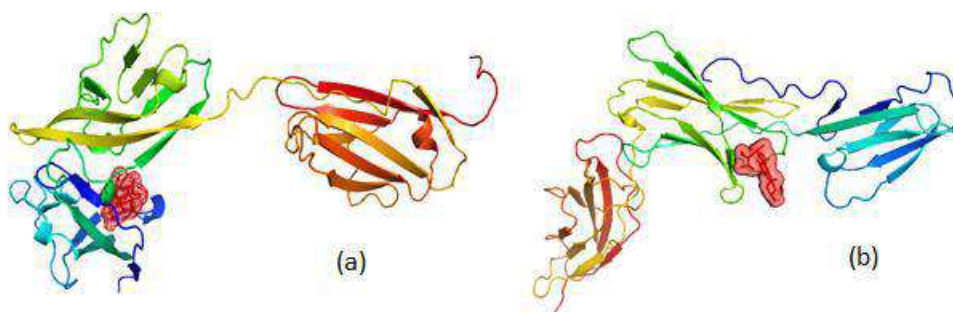


Figure 1. (a) and (b) are the structures of IL-1R and IL-6R- α bound to Stigmasterol at the chosen binding sites

The dried stem of the Venivalgata plant was used as one of the five ingredients in paspanguwa for its immense anti-oxidant, anti-inflammatory, and anti-cancer effects. The most abundant bioactive phytochemicals in *C. fenestratum* stem is Berberine, which possessed binding energies of -6.08 kcal/mol and -5.27 kcal/mol for IL-1R and IL-6R- α respectively. But the highest binding energy for IL-1R with a value of -7.74 kcal/mol was shown by Sitosterol glucoside. Other phytochemicals

such as, palmatine and berberrubine chloride, showed moderate amount of binding energies to both IL-1R and -IL-6R- α .

Table 1. Screening of Phytochemical Using Lipinski Rules and ADMET Predictions and their binding energies towards IL-6R- α and IL-1R [5]

No.	Compound	Lipinski Rule of Five		ADMET Predicted Profile		Binding Energies	
		Properties	Value	Property	Value	IL-6R- α (kcal/kJ)	IL-1R (kcal/ kJ)
1	6-Gingerol	No. of H bond doners	2	Carcinogenicity (Trinary) Human Intestinal Absorption Oral Bioavailability	0.7188	-5.49	-5.87
		No. of H bond acceptance	4		0.9924		
		Rotational Bonds	10		0.7714		
		ALog P	3.23				
		Molecular weight / mg	294.39				
		Violations	-				
2	Zingiberene	No. of H bond doners	0	Carcinogenicity (Trinary) Human Intestinal Absorption Oral Bioavailability	0.5088	-5.32	-6.59
		No. of H bond acceptance	0		0.979		
		Rotational Bonds	4		0.5571		
		ALog P	4.89				
		Molecular weight / mg	204.36				
		Violations	-				
3	β -Bisbolene	No. of H bond doners	0	Carcinogenicity (Trinary) Human Intestinal Absorption Oral Bioavailability	0.5271	-5.19	-6.38
		No. of H bond acceptance	0		0.9692		
		Rotational Bonds	4		0.5714		
		ALog P	5.04				
		Molecular weight / mg	20.36				
		Violations	1				
4	Camphor	No. of H bond doners	0	Carcinogenicity (Trinary) Human Intestinal Absorption Oral Bioavailability	0.6307	-4.46	-5.69
		No. of H bond acceptance	1		0.9779		
		Rotational Bonds	0		0.8774		
		ALog P	2.4				
		Molecular weight / mg	152.4				
		Violations	-				
5	Cineole	No. of H bond doners	0	Carcinogenicity (Trinary) Human Intestinal Absorption Oral Bioavailability	0.5599	-4.7	-4.98
		No. of H bond acceptance	1		0.9776		
		Rotational Bonds	1		0.7286		
		ALog P	2.74				-10.26

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		Molecular weight / mg	154.25				
		Violations	-				
6	γ-sitosterol	No. of H bond doners	1	Carcinogenicity (Trinary)	0.5888	-6.8	-10.26
		No. of H bond acceptance	1	Human Intestinal Absorption	0.993		
		Rotational Bonds	6	Oral Bioavailability	0.5286		
		ALog P	8.02				
		Molecular weight / mg	414.72				
		Violations	1				
7	Ursolic Acid	No. of H bond doners	2	Carcinogenicity (Trinary)	0.5962	-6.69	-9.15
		No. of H bond acceptance	2	Human Intestinal Absorption	0.9853		
		Rotational Bonds	1	Oral Bioavailability	0.5143		
		ALog P	7.09				
		Molecular weight / mg	456.71				
		Violations	1				
8	Oleanolic acid	No. of H bond doners	2	Carcinogenicity (Trinary)	0.5962	-6.29	-8.87
		No. of H bond acceptance	2	Human Intestinal Absorption	0.9853		
		Rotational Bonds	1	Oral Bioavailability	0.5429		
		ALog P	7.23				
		Molecular weight / mg	456.71				
		Violations	1				
9	Solasodine	No. of H bond doners	2	Carcinogenicity (Trinary)	0.4907	-6.25	-9.59
		No. of H bond acceptance	3	Human Intestinal Absorption	0.9721		
		Rotational Bonds	0	Oral Bioavailability	0.6286		
		ALog P	5.29				
		Molecular weight / mg	413.65				
		Violations	1				
10	Stigmasterol	No. of H bond doners	1	Carcinogenicity (Trinary)	0.5888	-6.95	-10.54
		No. of H bond acceptance	1	Human Intestinal Absorption	0.9914		
		Rotational Bonds	5	Oral Bioavailability	0.5571		
		ALog P	7.8				
		Molecular weight / mg	412.7				
		Violations	1				
11	Berberine	No. of H bond doners	0	Carcinogenicity (Trinary)	0.4717	-5.27	-6.08
		No. of H bond acceptance	4	Human Intestinal Absorption	0.7733		
		Rotational Bonds	2	Oral Bioavailability	0.5143		
		ALog P	3.1				

		Molecular weight / mg	336.37				
		Violations	-				
12	Sitosterol glucoside	No. of H bond doners	4	Carcinogenicity (Trinary)	0.682	-5.78	-7.74
		No. of H bond acceptance	6	Human Intestinal Absorption	0.6476		
		Rotational Bonds	9	Oral Bioavailability	0.7143		
		ALog P	5.85				
		Molecular weight / mg	576.86				
		Violations	2				

Conclusion and Recommendations

Traditional medicines like paspanguwa were more popular among local people of Sri Lanka for their therapeutic properties, contempt the absence of its scientific evidence. Paspanguwa has been used across many generations, as a remedy for many ailments such as, cold, fever, body ache with almost no side effects, when compared to western medicines. According to the results of this in-silico study, it is proven that the herbs in paspanguwa have shown tremendous results by binding to the receptors, IL-1R and IL-6R- α , inhibiting their activities of transmitting IL-1 and IL-6 inflammatory mediators, their-by performing an anti-inflammatory activity to the host. Phytochemicals such as Solasidine, Stigmasterol, Carpsterol, and Diosgenin in Katuwalbatu, Urosilic acid, and Oleanolic acid in Pathpadagam, zingiberene, and β -bisbolene in ginger, geraniol, and camphor in coriander and sitosterol glucoside in venvalgata, have shown the highest binding energies for both receptors, IL-1R and IL-6R- α .

Therefore, it is recommended to adjust the formulations according to studies, in order to get the maximum effectiveness of paspanguwa.

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THERMAL PROPERTIES OF RICE STRAW AND NATURAL LATEX COMPOSITES

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Introduction

Effective use of agricultural waste ameliorates energy conservation and environmental friendliness. This has drawn the attention of countries in which crop cultivation is their main revenue. Rice straw is a by-product after harvesting paddy. Each kilogram of milled rice roughly produces 0.7–1.4 kg of rice straw depending on the rice variety [1]. From ancient times, rice straw has been used as a building material for the roofing of houses to make the interior cool and comfort [2]. Rice straw is comprised of cellulose, hemicellulose, silica, and lignin. Cellulose and hemicelluloses are fibre organics, whereas lignin is in the cell wall of the biomass [1]. Therefore, in average natural rice straw comprises ~20% silica and ~80% carbonaceous material. Insulating buildings with good quality indoor air and proper circulation is a significant challenge for energy management in construction. Apart from this, the key role is played by the cost and the type of the insulation material when it comes to the application [2]. The novelty and principal motive of this study are to engineer eco-friendly composite material with low thermal conductivity.

The rice straw and the Natural Latex (NL) which are considered as materials with low thermal conductivity, cost-effective and abundantly found in Sri Lanka have been used in this present study to fabricate the eco-friendly composite, where straw was the base, and the NL was the binder.

Materials and Methods

Raw rice straw obtained from the field was cleaned and cut into small lengths and was oven-dried at 80 °C for 4 hours. These dried cut pieces of rice straw were then blended to get fine strands to make the composite samples. Varying weights of this blended straw were mixed with a constant 5 mL volume of NL having 60% of dry rubber content to make a series of composites, as shown in Table 1.

NL was chosen as the binder due to its availability, adhesiveness and high insulating properties. Prior to the addition, the NL was stirred at a temperature

of 70 °C with a stirring speed of 50-60 rpm in order to remove ammonia present in it [3].

Table 1. The ratio of straw and NL in the composites

Weight of straw (g)	Volume of NL (mL)
0.25	5
0.50	5
0.75	5
1.00	5
1.25	5
1.50	5
1.75	5

Two series of composites were made, one subjected to pressure and the other without pressure. The unpressurized composite was hand mixed and moulded to a sample of uniform thickness of 5 mm, ensuring that the NL well bound the straw. The other composite was mixed by a blender and then pressurized using the hydraulic pellet press with 5 Tons of force, making a sample of the same thickness of 5 mm. The prepared samples were oven-dried at 60 °C for 6 hours and then left out in the sunlight for two days to confirm the removal of moisture. This was ensured by weighing the samples at regular time intervals until a constant weight was observed for each sample. The thermal properties of the finally dried composite samples were measured using Hot Disk Thermal Constants Analyser Transient Plane Source (TPS) 500.

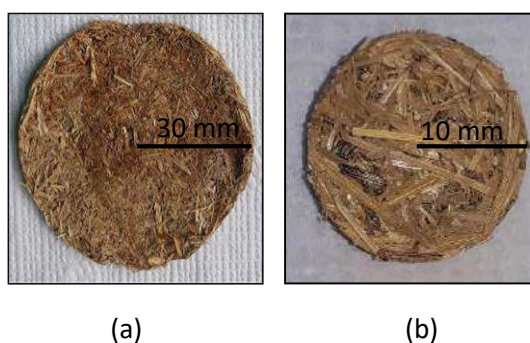


Figure 1. Straw composites (a) without pressure (b) with pressure

The TPS method is based on using a transiently heated plane sensor for the measurement of thermal properties. This method can measure the thermal conductivity, thermal diffusivity and volumetric specific heat capacity in a short time for isotropic materials over a wide range of temperatures. One significance of this analyser is that all three values could be obtained at one sample measurement. The Hot Disk sensor consists of an electrically conducting pattern

in the shape of a double spiral, which has been etched out of a thin metal (Nickel) foil. This spiral is sandwiched between two thin sheets of an insulating material (Kapton). When performing a measurement, a sensor is placed between two pieces of the measuring sample where the surface facing the sensor has to be smooth. An electrical current, strong enough to increase the temperature of the sensor between a range from a fraction to several degrees, is passed through it. At the same time, the increase of resistance (temperature) as a function of time is recorded simultaneously. Thus, making the Hot Disk sensor act both as a heat source and a dynamic temperature sensor. The solution of the thermal conductivity equation is based on the assumption that the Hot Disk sensor is located in an infinite medium, which means that the transient recording must be interrupted as soon as any influence from the outside boundaries of the two sample pieces is recorded by the sensor. Here, the samples were measured at room temperature under the parameters of heating power 50 mW and measuring time of 10 s using the Kapton 5465 sensor.

Results and Discussion

The change in the thermal conductivities of both unpressurized and pressurized samples with different composites made of straw content of 5%, 12%, 17%, 21%, 25%, 30%, 32% was observed, as shown in Figure 2. Both samples follow the same pattern in their results when the weight of the rice straw increases. It is seen clearly that the thermal conductivity values drop and then rise with the increase in the weight of straw. The minimum value is observed when the straw content weight percentage is closer to 25 in both cases. This shows a significant decrease in thermal conductivity when increasing the amount of rice straw in the composites until it reaches around 25% which is the critical composition. An increase in thermal insulation property of ~71% compared to the composite with the lowest weight of rice straw is noticeably seen in the unpressurized composite in Figure 2. The unpressurized sample's thermal conductivity with the 25% weight of straw exhibited a lesser value than the values obtained for the pure latex and pure straw, which are $0.1588 \text{ W m}^{-1} \text{ K}^{-1}$ and $0.0954 \text{ W m}^{-1} \text{ K}^{-1}$, respectively, which is an interesting observation in this study.

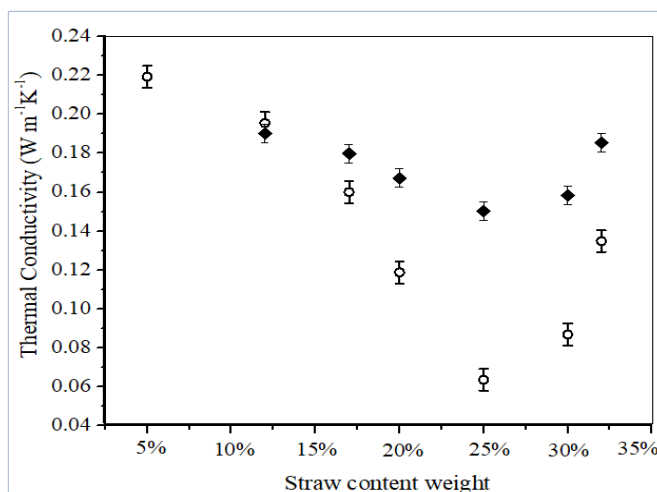


Figure 2. Thermal conductivity of the pressurized (◆) and unpressurized samples (○)

This could be due to the crosslinks of latex macromolecules and three-dimensional network giving a unique property to the composites comparably to the other samples. Further, the disorientation of the straw trapping more air spaces could have prevented continuous heat transfer throughout the sample. After the critical composition, the thermal conductivity shows an increase with the weight of the straw. This could be due to the straw's dominating effect taking over the composite effect. The silica and other materials which support the thermal conducting property present in the strands could give a cumulative contribution to this behaviour, inhibiting the insulation effect. Apart from this, the penetration of air through the composite may be inhibited with the increase in the weight of straw. Yet further investigation should be done to understand this behaviour.

Figure 2 also shows the plot of the thermal conductivity of composites made out of the same sample composition but pressurized under 5 Tons of force using a hydraulic pellet press. Their results also show the same trend as in the previous scenario but higher in thermal conductivity. However, its value when the straw content is around 25% was found to be very much higher than the sample made without pressure. This could be due to the drastic decrease of air spaces while compressing and tightening the straw and latex. Another notable feature observed overall is that at the regions where the lesser content of the weight of straw and higher content of the weight of straw, the values of the thermal conductivities seem to converge.

Table 2. Thermal conductivity of different thermal insulation materials

Samples	Thermal conductivity ($\text{W m}^{-1} \text{K}^{-1}$)	Source
Particle board from mixture of durian peel and coconut coir	0.0728	[4]
Straw and NRL composite	0.0636	Present work
Insulation board from water hyacinth fiber with NRL	0.0246	[3]
Insulation board from pineapple leaf fiber with NRL	0.0223	[5]

Table 2 shows the comparison of the fabricated composite with that of other thermal insulation materials. Interestingly, the rice straw with NL had a lower thermal conductivity range than that of particleboard from the mixture of durian peel and coconut coir and is in the order with the other two thermal insulation materials mentioned in Table 2. This shows that the rice straw with NL could be considered as one of the suitable insulating materials for industrial purposes. Work on fabrication and analysis for pressurized composite is under progress for further study. Since this is a preliminary work, the exact cost for market cannot be deduced at this stage.

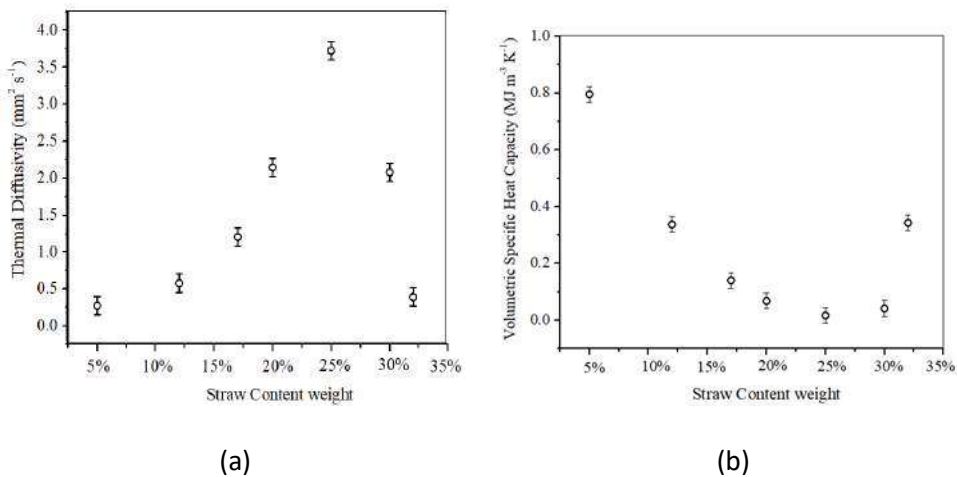


Figure 3. (a)Volumetric Specific Heat Capacity (b) Thermal Diffusivity of the unpressurized composites

A constant small amount of NL was used to bind the straw strands. Adding more NL in the samples tended to increase their thermal conductivity, which may be due to reducing the air gap between the straw strands resulting in the high heat transfer. This observation agreed with that of other workers [3,4,5].

Apart from the thermal conductivity, volumetric specific heat capacity and thermal diffusivity were obtained simultaneously for unpressurized samples. Figures 3(a) and 3(b) show the variation of the respective samples' volumetric specific heat capacity and thermal diffusivity. The study of these two properties for pressurized samples are in progress for comparison and interpretation. Further investigations are needed to realize the mechanism of thermal conduction, thermal diffusivity and volumetric specific heat capacity in the composite material considering its composition.

Conclusions and Recommendations

The principal motive of this study is to be engineering a higher insulating thermal composite combining two low thermally conducting materials and study its thermal properties. With the increase of the weight of straw, the thermal conductivity was observed to be decreasing in both pressurized and unpressurized composites. The lowest value of $0.0636 \text{ W m}^{-1} \text{ K}^{-1}$ was observed for the unpressurized composite sample with straw content of 25%. This composite further showed an increase in thermal insulation property of ~71% compared to the composite with the lowest weight of a straw in unpressurized samples. Increasing further content of straw, the thermal conductivity increases as the cumulative effect of silica appears and less air penetration. Henceforth, further work could be planned on this economical and eco-friendly composite for industrial applications.

Acknowledgement

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ANTIBACTERIAL ACTIVITY OF A HERBAL DEODORANT FORMULATED WITH *Nymphaea pubescens* FLOWER PETALS AGAINST ISOLATED HUMAN SKIN MICROFLORA

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Introduction

Deodorants are personal hygienic products that are used to avoid distinctive malodorous scents, simple “body odour” of an axillary area of the human body. The body odour is caused by the interaction of the resident bacteria with the apocrine sweat and armpit microbial communities are mainly dominated by Staphylococcaceae [1]. Therefore, deodorants and antiperspirants are basically formulated with antibacterial activity against pungent odour causing bacteria and with synthetic fragrances such as acetyl cedrene and lylal (3- and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-aldehyde). However, certain individuals can develop allergic contact dermatitis and some other adverse effects on skin by application of chemical- based deodorants [2]. For that problem, herbal deodorants with no added synthetic antimicrobials and fragrances would be a great alternative. In the present study, we investigated the *in vitro* effect of a newly formulated *Nymphaea pubescence* flower petal extract-based deodorant on *Staphylococcus aureus* and a coagulase-negative *Staphylococcus* sp. isolated from human skin microbiota.

Materials and Methods

Identification of isolated bacteria

Skin microflora obtained from skin swabs and cultured on Mannitol Salt Agar (Oxoid, UK) was available at the Faculty of Medicine, University of Ruhuna and was employed for this study. The isolates were subcultured separately on Mannitol Salt Agar according to the colour of the colonies to obtain purified bacteria. After incubation, each purified isolate was inoculated on Nutrient Agar (Oxoid, UK) and incubated at 37 °C for 24 hours. Then isolates were observed after Gram staining using a microscope. Confirmed colonies of *S. aureus* and coagulase-negative *Staphylococcus* sp. were selected.

Evaluation of the antibacterial activity of the herbal formulation

The antibacterial activity of *N. pubescens*-based formulation was assessed using a modified broth micro-dilution method [3] against both *S. aureus* and coagulase-negative *Staphylococcus* sp. isolated from the human skin. Four concentrations of the formulation (0.5%, 1.0%, 2.0%, 2.5% and 5.0%) diluted with sterile distilled water were initially tested for antibacterial activity. After the incubation at 37 °C for 24 hours, a loopful of each well was sub-cultured on Nutrient Agar medium for screening for viability. Standard ciprofloxacin was used as the positive control and sterile distilled water was used as the negative control. The experiment was performed in duplicate.

Results and Discussion

The initial screening revealed potent antibacterial properties in all four concentrations of the deodorant formulation against both *S. aureus* and coagulase-negative *Staphylococcus* sp. Thereafter minimum inhibitory concentration (MIC) of this *N. pubescens*-based deodorant formulation was determined and the results are summarized in Table 1

Table 1. Minimum Inhibitory Concentrations (MICs) obtained for isolated bacterial strains

Bacterial Strain	Minimum Inhibitory Concentration (MIC) (mg/mL)	
	Formulation	Positive Control
<i>S. aureus</i>	0.08	0.032
Coagulase- negative <i>Staphylococcus</i> sp.	0.032	0.013

No inhibition was observed in negative controls.

The obtained results revealed that the formulation is capable of inhibiting the growth of skin inhabiting bacteria. *N. pubescens* leaf and flower extracts have been reported to possess antibacterial potential against several bacterial species including *Staphylococcus aureus* [4]. However, to the best of our knowledge, this is the first report on the antibacterial potential in a deodorant formulated from the hydroalcoholic extract prepared from flowers of *N. pubescens* against skin microbiota.

Conclusions and Recommendations

N. pubescens flower-based deodorant has the potential to inhibit the growth of *S. aureus* and coagulase-negative *Staphylococcus* sp. isolated from human skin. Therefore, it has a great potential to be developed into an herbal-based deodorant in commercial scale.

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IN-SILICO STUDY ON FEASIBILITY AND STABILITY OF HUMAN URACIL DNA GLYCOSYLASE WITH ARSENIC(III)

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Introduction

Human uracil DNA glycosylase (hUNG) removes uracil in DNA by initiating the base excision repair pathway and is considered as a DNA repair enzyme. These enzymes can be affected by the accumulation of toxic heavy metals. Some studies that have been done on heavy metal accumulation on hUNG have reported that the cadmium(II) has the potential to inhibit the activity of hUNG by replacing the catalytic water found in the active site of the enzyme and forming close contacts with some residues in the active site region of the enzyme [1]. However, there is not much evidence on the accumulation of heavy metal ions other than Cd(II) on the hUNG enzyme. Therefore, the present study focuses on arsenic, also categorized as a toxic metal on the hUNG enzyme. This work aims to study the feasibility of As(III) to bind with hUNG and compare the stability of the free enzyme and the enzyme after the binding of As(III). The work is based on computational analysis using the simulated trajectories of the free hUNG system and the enzyme system with As(III). The formations of the hydrogen bonds of the enzyme in the two systems were studied using the ProteinTools web server. The secondary structure analysis based on the Ramachandran plot was studied using the PROCHECK server.

Materials and Methods

The crystal structure of the hUNG enzyme was downloaded from the protein data bank (PDB ID: 1AKZ, Resolution: 1.57 Å). Then, two systems of the enzyme; one with the free hUNG and the other with hUNG and As(III) were prepared to perform the MD simulations using GROMACS software. The protein topology was generated using the Kirkwood Buff force field (KBFF). A cubical-shaped simulation box was generated, placing the free enzyme at the center of the box, maintaining a minimum distance of 1.5 nm between protein and the edge of the simulation box. The simulation box was then solvated with SPC/E water. The electro-neutrality of the system was maintained by adding five chloride ions. The system was then subjected to 3000 steps of energy minimization using the steepest descent algorithm followed by a 100 ps equilibration step. Next, the equilibrated system was allowed for 100 ns MD simulation maintaining the temperature and the pressure of the system at 300 K and 1 bar, respectively, using Nose-Hoover

thermostat and Parrinello Rahman barostat. The same procedure was followed in preparing the hUNG-As(III) complex system. Based on the published reports, As(III) was placed by replacing the catalytic water in the enzyme's active site. The electro-neutrality of the hUNG-As(III) complex system was maintained with the addition of eight chloride ions. The Lennard-Jones parameters; σ and ϵ As(III), 0.423 nm, and 1.2928 kJ/mol, respectively, were taken from the published work [2]. The enzyme's hydrogen bonds formed in the two systems were analyzed using the ProteinTools web server [3]. The binding free energy of As(III) with hUNG was calculated using molecular mechanics poisson Boltzmann surface area (MMPBSA) method for the metal ion and enzyme in the final structure [4]. The final PDB structures of the enzyme in the two simulated systems were used to analyze the stability change in protein secondary structure using the PROCHECK server [5].

Results and Discussion

The MMPBSA method calculated negative binding energy for As(III) with hUNG, and the binding free energy was given as -358.152 ± 16.078 kJ/mol. The negative sign of the energy denotes the feasibility of As(III) to bind with the enzyme.

The ProteinTools web server identified ten hydrogen bond networks with twenty-one hydrogen bonds from all networks for the free enzyme hUNG. The enzyme system with As(III) was identified with sixteen hydrogen bond networks with twenty-four hydrogen bonds from all the sixteen hydrogen-bond networks. Figure 1 shows all the hydrogen bonds formed within the enzyme in the two systems of the enzyme. According to the server results, the enzyme in the presence of As(III) shows the highest number of hydrogen bonds than the free enzyme. This implies that the enzyme gets stabilized in the presence of As(III), and the stability is higher than the free enzyme due to the formation of more hydrogen bonds within the enzyme.

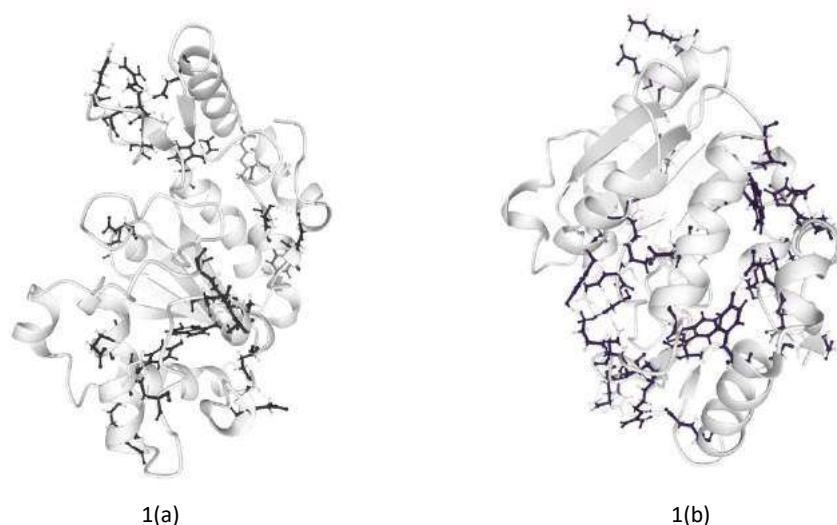


Figure 2. All the hydrogen bonds identified by the ProteinTools web server within the enzyme in the (a) absence of As(III) and (b) in the presence of As(III).

The PROCHECK server gives the stereochemical quality of a protein structure. The server interprets the results using the Ramachandran plot. Figure 2 (a) shows the Ramachandran plot obtained for the 100 ns simulated free enzyme, while Figure 2 (b) represents the result of the enzyme in the presence of As(III). The results show that a percentage of 89.4 of residues lie in the allowed region of the Ramachandran plot for the enzyme with As(III) while 84% of residues for the free enzyme. Therefore, a high percentage of residues can be observed in the allowed region of the Ramachandran plot for the hUNG with As(III) than the free enzyme.

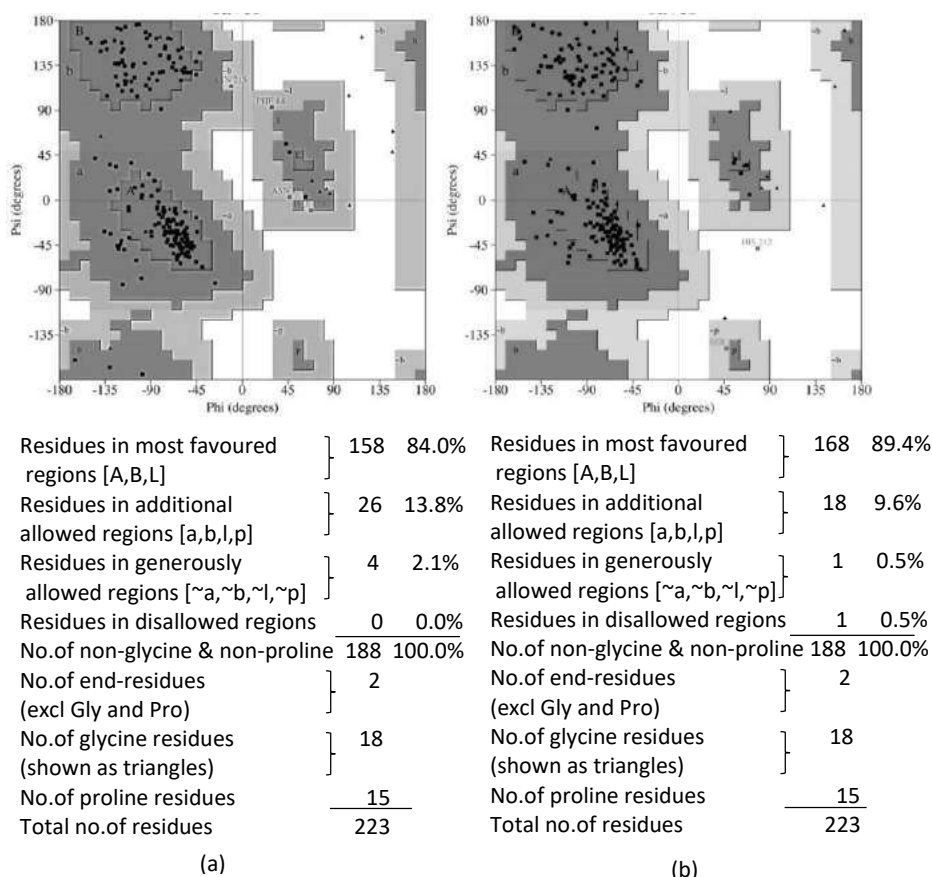


Figure 3. Ramachandran plot for the free (a) hUNG and (b) hUNG with As(III)

Conclusions and Recommendations

The binding free energy between the enzyme and As(III) denotes the feasibility of As(III) to bind with the enzyme hUNG. The negative binding free energy indicates that As(III) can bind with the enzyme, and the process is energetically favored. Therefore, the binding free energy results show that the As(III) can bind with the enzyme hUNG.

The results of the ProteinTools web server showed that the number of hydrogen bonds formed within the enzyme gets reduced for the free enzyme comparative to the enzyme in the presence of As(III). Since the formation of more hydrogen bonds results in a well-stabilized system, the enzyme in the presence of metal ion can be considered the most stable enzyme than the free enzyme.

The effect of As(III) binding on the protein structure can be determined through the Ramachandran plot by identifying the torsional angles of the residues. Considering the percentage shown in the most favored region in the Ramachandran plot, the residues in the enzyme with As(III) show the highest percentage (89.4) compared to the free enzyme (84%). This indicates that the residues of the enzyme in the presence of As(III) obtain a stable spatial arrangement than the residues in the free enzyme. Hence, the enzyme gets structurally stable in the presence of As(III).

Therefore, this study concludes that the binding of As(III) with the enzyme hUNG is feasible and forming an enzyme-metal ion system is more stable than the free enzyme.

Acknowledgement

The World Bank and the AHEAD Operation are acknowledged for providing funds for this study.

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FOCUS AREA

Energy

PRODUCTION OF BIODIESEL FROM OLEAGINOUS YEAST (*Lipomyces Starkeyi*) BY USING BIO-CONVERSION OF HYDROLYSATE CORNCOB

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Introduction

The majority of the world's energy needs are supplied through petrochemical sources, coal and natural gases, with the exception of hydroelectricity and nuclear energy, of all, these sources are finite and at current usage, rates will be consumed shortly [1]. A unique solution to the shortage of petroleum fuels is the production of biodiesel from lignocellulose materials. Compared with traditional fossil fuels, lignocellulose materials, as a bio-mass energy source, are land-based biomass instead of fossil energy deposits, processing, and conversion of carbohydrates instead of hydrocarbons. Corncob has consequently become abundant lignocellulose bio-mass from agriculture. Lignocellulose biomass is primarily composed of the two carbohydrate polymers, cellulose and hemicellulose, and the non-carbohydrate phenolic polymer, lignin. Lignin binds to cellulose fibers to harden and strengthen the plant cell walls. In general, LB contains 38–50% cellulose, 23–32% hemicellulose, and 15 – 25% lignin shown by. The components of corncob are hemicellulose, cellulose, and lignin 36.4%, 38.8%, 13.1% [2]. Hemicellulose hydrolysate has been used directly to cultivate oleaginous yeast and yield microbial lipids, which were transesterified into biodiesel in situ.

Oleaginous yeasts can be introduced as cell factories for sustainable production of microbial lipids by the fermentation of agricultural wastes as the lignocellulose material. Oleaginous microorganisms such as yeasts, microalgae, and bacteria, that microorganism can accumulate oil 20% of its dry weight and can reach up to 60 – 70% in some cases. In this present study, Corn-cob is used as the lignocellulose material. Corn-cob is used as the substrate to accumulate to the microbial lipid by using *Lipomyces Starkeyi* as oleaginous yeast. *Lipomyces Starkeyi* is the most extensively studied yeast, chosen for its excellent ability to produce lipid [3].

Materials and Methods

The Corncob was collected in a local area in the Anuradhapura, Galagmuwa, North-Western province, Sri Lanka. The corncob was washed with running water and air-dried. Dried corncob was ground into less than 1 mm size by using an electric flour grinding mill. 2.5% sulfuric acid was used to pretreat the corn cob, the corncob was mixed with 2.5% sulfuric acid with a 1:10 ratio (w/v) and

autoclaved in an autoclave reactor at 121 °C for 1 hour. The corncob was mixed with 2.5% sulfuric acid with a 1:10 ratio (w/v) and autoclaved in an autoclave reactor at 121 °C for 1 hour. The hemicellulose hydrolysate and residue were extracted through vacuum filtration. Hemicellulose hydrolysate was analyzed using HPLC [4]. 3% activated carbon was added to the hemicellulose hydrolysate until mixed well by using a magnetic stirrer at 40 °C for 1 hour at a shaking speed of 200 rpm, then vacuum filtrated at 60 °C to remove the insoluble material. The commercially purchased NCYC 732 *Lipomyces Starkeyi* was cultured in YPD Agar media. 250 ml of hemicellulose hydrolyzate was added to each four 500 ml conical flask. *L.Starkeyi* has inoculated to each flask 625 mg, 1250 mg, 1875 mg, 2500 mg respectively and pH was adjusted to 4.9 pH (*L. Starkeyi* optimum pH value was 4.9 pH). Then it was kept in a rotary shaker incubator at 27 °C and 160 rpm for fermentation. Then, 15 g of hemicellulose hydrolyzate was taken from each sample at regular 12-hour time intervals of 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, 96 hours, 108 hours, 120 hours respectively for ensuring lipid accumulation. 1 liter of hemicellulose hydrolyzate was prepared by using the above method and it was fermented according to the best parameters above method measured biomass and single cell oil (SCO) productivity. 20 mL of oleaginous yeast strain was extracted to the 250 mL conical flask and it was up to 160 °C temperature at 200 rpm and shacked by using a magnetic stirrer in 10 minuts. Then 0.01 M 2 mL of sulfuric acid and 150 mL of methanol were added to the yeast strain and the conical flask was sealed by using aluminum foil and kept at 160 °C and 200 rpm for 60 minutes. Yeast strain was poured into the separatory funnel for separating to the fatty acid methyl ester (FAME) and glycerol by the yeast strain. After 20 minutes, Glycerol was removed by carefully released the tap. Finally, the upper layer was extracted as the FAME (biodiesel). The composition of the concentrated FAME sample was detected by gas chromatography using a flame ionization detector, capillary column, and nitrogen as carrier gas. FAME composition was identified by comparing the retention time (RT) with authentic standards and quality was compared with ASTM standards [5].

Results and Discussion

HPLC test was done to analyze components of hydrolyzate before detoxification and after detoxification. After the detoxification process, inhibitors of hydrolyzate were detected 0.02 g/l of 5-hydroxymethylfurfural.

Table 1. Composition of the corncob hydrolysate before and after detoxification

Compound	Before detoxification	After detoxification
Xylose (g/l)	41.62 ± 0.20	36.79 ± 0.43
Glucose (g/l)	8.83 ± 0.56	7.22 ± 0.11
5-hydroxymethylfurfural	0.51 ± 0.04	0.02 ± 0.001
Furfural (g/l)	0.13 ± 0.02	0
Acetic acid (g/l)	3.23 ± 0.15	0

The HPLC test was mainly targeted to identify components and inhibitors in the corncob hydrolysate. As shown in Table 1, xylose was the main component in corncob hydrolysate and it was measured as 41.6 g/l. 8.83 g/l glucose was in a corncob hydrolysate. Three inhibitors were detected including 5-hydroxymethylfurfural, furfural, and acetic acid in the corncob hydrolysate. The HPLC test was performed again after the detoxification process. Then could be recognized low inhibitors percentage of the corn-cob hydrolysate, detoxified material was reacted with the inhibitors.

Table 2. Properties of biodiesel made from *Lipomyces Starkeyi*

Property	Value
Ash content	0.002 – 0.01 wt%
Cetane number	46 – 70
Density at 15 °C	820 – 860 kg/m ³
Flashpoint	135 – 150 °C
Higher heating value	39.3 – 39.8 MJ/kg
Sulfur content	0.0000 – 0.0024 wt%
Viscosity at 40 °C	3.7 – 45.8 mm ² /s

Fatty acid methyl ester characteristic is the analysis by using Gas Chromatography and biodiesel properties such as Gravimetric analysis, Specific gravity, Higher heating value, Cetane number, Viscosity, Cloud point, Cold filter plugging point (CFPP), Iodine value are measured by using several methods. Gas Chromatography test was detected myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linolenic acid were 16.7%, 24.4%, 1.4%, 2.9%, 46.8%, and 6.5%(V/V) respectively in the FAME sample.

Conclusions and Recommendations

The expected outcome of this study is that the corncob hydrolysate could be used as the feedstock for accumulating the SCO from the *Lipomyces Starkeyi*. Approximately, 60% microbial lipid could be expected by introducing *Lipomyces Starkeyi* yeast strain. The major fatty acids were shown as long-chain fatty acids (C16-C18) and the extracted oil from the yeast strain *Lipomyces Starkeyi* could be

effectively converted into biodiesel. Furthermore, it could be expected that the oil extracted from the *Lipomyces Starkeyi* yeast strain could be effectively converted into biodiesel (FAME) which can be introduced as a common solution to the current energy crisis. I expected that, by improving the biodiesel production with wastage, we will be able to maximize the usage of FAME for our energy consumption about 30% in the future. By enhancing the properties of biodiesel, it can be used directly for transportation, power generation, agriculture, and various other fields. Greenhouse gas emissions, dependence on foreign suppliers, and the use of fossil fuels can be minimized with this solution.

Acknowledgment

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LOW-COST MACROPOROUS LAYER FOR GAS DIFFUSION ELECTRODE

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Introduction

The electrochemical reduction of CO₂ (ERC) is an encouraging technology among the various strategies that have been presented to lessen the concentration of Carbon dioxide (CO₂) in the atmosphere. ERC is the process of reducing CO₂ to more usable chemicals, particularly "CO₂ neutral fuels," using electrical energy (also known as artificial photosynthesis). It simultaneously offers remedies to two modern issues. They are CO₂-related global warming and renewable energy storage. The ERC occurs at the interface of an electron conductor (cathode) and an ionic conductor in an electrochemical cell (electrolyte). Water oxidation at the anode and CO₂ reduction at the cathode are both involved in the ERC process (Figure 1.). Many researchers have declared that ERC in large-scale use is economically feasible [1,2]. Since it uses less energy than typical chemical reduction processes and can operate at a moderate temperature and air pressure. ERC's economic feasibility is heavily dependent on the cathode, which includes its electrochemical performance and cost.

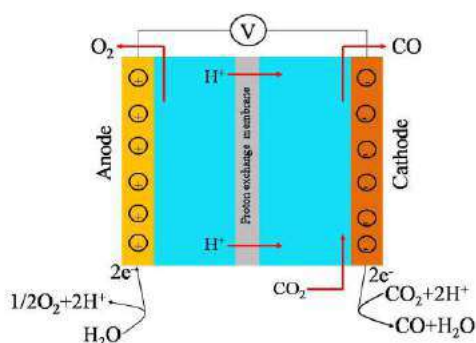


Figure 1. Electrochemical cell for CO₂ reduction

Because the majority of existing ERC cathodes are metal electrodes, CO₂ is sparged in the bulk. The mass transfer of CO₂ from the bulk to the cathode surface limits the efficiency of the ERC process since CO₂ solubility in water under ambient conditions is relatively low (0.033M). Gas diffusion electrodes (GDEs) have been added to the ERC system to improve its efficiency.

GDE is a porous composite electrode, usually consisting of a gas diffusion layer (GDL), a current collector (CC), and a catalyst layer (CL) (Figure 2.). The GDL has double functions of gas delivery and waterproof. The CC is used to reduce the electrode ohmic losses. The CL possess gas-liquid-solid three phase interface (TPI), which is the reaction site of ERC. This heterogeneous interface provides high number of CO₂ to the reaction than in a normal electrode where there is homogeneous interface and limited diffusion. It has been detailed that the productivity of ERC can be expanded by one to two orders of greatness by utilize of GDEs.

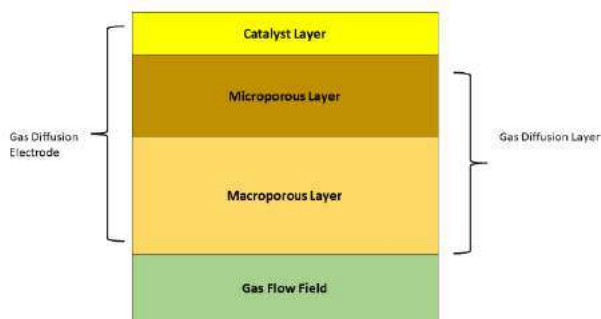


Figure 2. Structure of a gas diffusion electrode

There are two types of GDLs, single layer and dual layer GDLs. Dual layer GDE are frequently used, microporous layer is combined with macro-porous layer or macro-porous substrate (MPS). The microporous layer is top of the macro-porous layer consists of carbon and hydrophobic agent (usually PTFE). Microporous layer has direct contact with gas field and act as a current collector and a gas diffuser.

A macro-porous layer is usually made from carbon fiber-paper, carbon paper and carbon foam [5]. Carbon is used as a MPS due to its high electric conductivity, elasticity under compression. Various additives like polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), and fluorinated ethylene propylene (FEP) have been used for hydrophobic treatments of the carbon paper surface to control the wettability of the MPS. The other type is metal-based MPS such as metal mesh and metal foam. They have been extensively investigated due to their excellent mechanical strength. It provides a physical support to the catalyst layer. Here, metal mesh used as the current collector.

Microporous layer (MPL), usually fabricated by mixing hydrophobic agent PTFE with carbon black powder [3,5]. MPL is located between the catalyst layer and macro-porous layer. The purpose of the MPL is to maximize the electrical conductivity and help to improve the water management as it provides effective water transport. Many researchers have been investigated about MPL and MPS.

Paganin et al [3] observed that there is slowly increase in resistance with the thickness (35-65 μm) of the GDL where carbon cloth has been used as the substrate and explained it can be happened due to flooding problem. Too thin GDL will not have sufficient amount of PTFE and carbon black to produce good electrical resistance. Erkan et al [4] investigated the effects of GDL characteristics and pore-forming substances on the performance of PEMFCs using four types of carbon paper GDLs, GDL 30, 31, 34, and 35 BC (Sigracet1), and carbon cloth GDLs (EAE). They observed most of the SGL carbon paper GDLs performance was higher than the carbon cloth GDL. Williams et al [5] have been characterized and compared the properties of five commercial GDLs and in-house GDL. In house GDL showed lower electronic resistivity (0.0043 Ωcm) than commercial GDLs. Highest electronic resistivity value of 0.02 Ωcm obtained for SGL_10BB GDL. Those results showed that electronic resistivity of dual layer GDL depend on MPS.

The MPL needed to provide proper pore size and hydrophobicity to avoid flooding in water management and to increase the electrical contact with the catalyst layer. The optimum content of 20 wt % has been found at high current density values by researchers. Optimum concentration of PTFE is needed for the effective removal of product water to improve the cell performance. Liquid contact measurement is used for quantitative study of wetting ability solid surface by liquid. In water management with the application PEFC, contact angle is an important parameter for characterize the properties.

On the basis of the information discussed above, the aim of this work is to fabricate economically feasible GDE as the stainless mesh is not expensive like carbon cloth and carbon paper. For that purpose, the influence of the substrate of GDLs were studied. To achieve this goal, fabricated MPLs on three different substrates were characterized by measuring contact resistance and contact angle.

Materials and Methods

Materials

Vulcan XC 72R Carbon Black Powder (bulk density 96 kgm^{-3}), PTFE DISP 30 (TE3970 type), Toray carbon paper 090/MPL and Freudenberg H23C6 (carbon fiber paper) were purchased from Fuel Cell Store, USA. Commercially available Ethanol 95%, and stainless- steel mesh was used without further treatment.

Methods

Fabrication

First the mixture of conductive carbon black and ethanol have been agitated ultrasonically. Then PTFE has been added and further agitated ultrasonically and stirred in a hot bath. The mixture has been stirred in ultrasonic bath during the whole procedure to remove the air trapped inside the extremely porous carbon

particles improving wettability and homogeneity. The dough has been formed rolled down to a thin film on carbon paper, Freudenberg H23C6 and stainless-steel mesh separately. The Fabricated GDL is shown in Figure 3. After that it has been sintered to form a gas diffusion layer.

For the preparation of MPL, carbon black loading 25 mgcm⁻², 20% PTFE and 5 ml of ethanol were used for each of the GDLs.

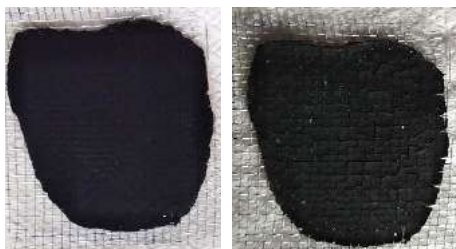


Figure 3. Fabricated microporous layer on stainless steel mesh (macro-porous layer)

Characterization

Contact Resistance: Contact measurement was carried out placing the sample between two copper plates under constant pressure, at room temperature. Voltage and current measurements were taken by KEITHLEY 2400 Source Meter. Material resistivity was measured by the following equation (1)

$$\rho = R \cdot \frac{A}{l} \quad (1)$$

where ρ is material resistivity (Ωcm), R is the resistance (Ω), A is the cross-section (cm^2) ($2\text{cm} \times 1\text{cm}$) and l is the thickness of the micro-porous layer (cm).

Contact Angle: The water contact angle of the GDLs were measured using the sessile drop method. In this method, the contact angle of the water droplet on the solid surface of the GDL was measured using ImageJ software (open source). The water contact angle was measured for fixed volume droplets of 5 μL at three points on the surface of the GDL.

Results and Discussion

Contact Resistance

The calculated resistivity for different substrates (MPS) is shown in Figure 4.

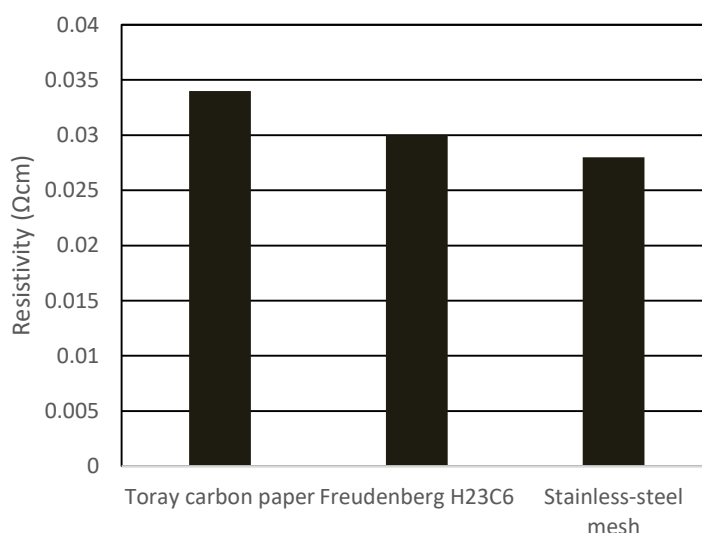


Figure 4. Electronic resistivity for different substrates

Among all the GDLs, Stainless-steel has the lowest resistivity of 0.028 Ωcm and highest resistivity of 0.034 Ωcm for Toray carbon paper. The overall resistivity of GDLs were reduced when MPL is added. GDL where Toray carbon paper used as the MPS has low electronic resistivity of 0.034 Ωcm to the high resistivity of bare Toray carbon paper used as its MPS being 0.08 Ωcm. GDL made by Freudenberg H23C6 showed a in between value of electronic resistivity. There is no huge variation in obtained results for three GDLs. Stainless-steel also showed good characteristics compared to standard MPSs.

GDL with lower resistivity is more effective as a current collector. Electron transport through gas diffusion layer is affected by electrical resistance as electron flow reduce with increasing resistance.

Contact Angle

GDL has an important role in water management. The surface water contact angle depends on hydrophobicity of material and the roughness of the surface Contact angle measurement is important for understanding the interaction of gas diffusion material with water. In this research work hydrophobic (water contact angle > 90°) GDL surfaces were observed. The values obtained here is within the range 100° and 120° for the dual layer GDLs. Toray carbon paper has a PTFE treatment of 8-9% and MPL has a PTFE content of 33-35% and Freudenberg H23C6 also a carbon GDL with MPL as well as a hydrophobic treatment. PTFE treatment to the micro-porous layer make it more hydrophobic and it will limit the water penetration (wet-proof) in to the GDL.

Table1. Values of left/right angles for different substrates

Substrate (MPS) with MPL	Left angle (°)	Right Angle (°)
Toray carbon paper 090	118	117
Freudenberg H23C6	118	119
Stainless-steel mesh	112	108

Conclusions and Recommendations

Characterization of three different GDLS have been done in the research work. The electronic resistivity and contact angle measurements were compared with each other. Contact angle measurement were in the range of 100° to 120°, therefore the fabricated GDLS are hydrophobic. The obtained results showed that electronic resistivity is depend on the MPS. Among the GDLS, GDL prepared using Toray carbon paper showed the highest electronic resistivity value of 0.034 Ωcm and lowest resistivity of 0.028 Ωcm was obtained for stainless steel mesh. Since there is no a huge variety within the results, stainless-steel mesh can be used to fabricate GDLS because it is cost-effective and it is readily-available in the local market. Results Optimization of GDE fabrication is to be done as future work.

Acknowledgment

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FOCUS AREA
Environment

OROGRAPHIC EFFECT ON THE HEAVY PRECIPITATION, FLOOD DISASTER AND TROPICAL STORM IN MAY 2017 IN SRI LANKA

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Introduction

Sri Lanka receives rainfall throughout the year from first inter monsoon in March to April, South West monsoon in May to September, second inter monsoon in October to November and North-East monsoon in December to February. Thunderstorms and deep depression over the Northern Indian Ocean bring rainfall. Indian Ocean dipole, air pollution and global warming increase the occurrence of extreme rainfall events in Sri Lanka. Heavy rain trigger spates of landslides and floods in the wet zone. The South-west monsoon normally brings about 200mm - 3000mm. In this case, Sri Lanka experienced notably heavy rainfall in the latter part of May 2017. Over 0.5 million people were affected and over 230 people were killed by the flood and landslides. During the 24-hour period on 25th May the recorded rainfall was about 300-500 mm where Rathnapura received nearly about 453 mm of highly accumulated rainfall. Continuous rainfall leads to floods and landslides across the region. At the end of May 31, the deep depression further enhanced into cyclonic storm “Mora” in the Bay of Bengal. The majority of the casualties resulted from landslides which destroyed numerous homes of Matara, Ratnapura and Kalutara districts. Apart from the heavy monsoon, an enhanced extremely rainy season that preceded the slope failure escalated the landslide events. Climate extreme rainfall events were favored by the continuous supply of moist air, air instability and buoyancy creating convection zone and deep convection by Madden Julian oscillation (MJO) phases 3 and 4 over the Northern Indian Ocean [1]. Moisture emerge from the sea surface is source for rainfall on land [2]. Because Sri Lanka is prone to tropical extreme climate events natural disasters claim lives and property. This study is conducted to examine the plausible projection for orographic effect on localized record-breaking precipitation, floods and landslides in May 2017 in Sri Lanka. Anchor shaped Mountain ranges of central high lands are traverse from North to South for 120 km. With the horizontal westerly winds blow perpendicular to the mountain slopes orographic effect is enhanced. Preliminary investigation of this study suggests that torrential rain with large-scale atmospheric precipitation moisten and weaken the soil slopes with predecessor condition.

Materials and Methods

Data

Data for the analysis were acquired from the NOAA and NASA archived data stores. Daily rainfall data for the period of 24th to 27th of May 2017 was obtained from the NOAA IRI site. Windgram index for the period was obtained from NASA READY site. An elevation map of Sri Lanka was accessed from Zubair 2003. Integrated water vapor transfer and wind speed were also utilized from NASA MERRA 2 GIOVANNI store.

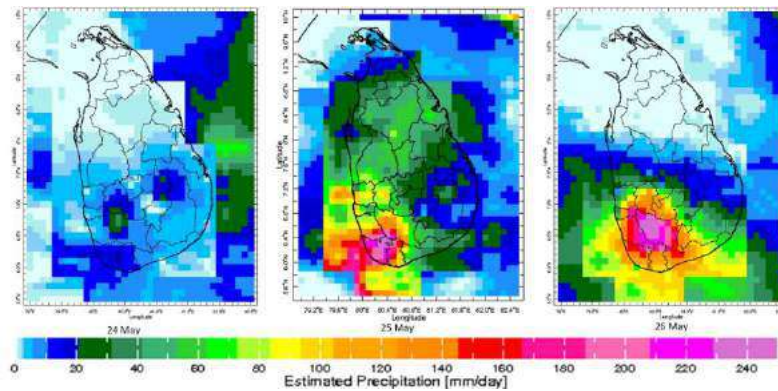


Figure 1. Daily Rainfall variation over Sri Lanka for the 3-consecutive days from 24 May to 26 May 2017

Figure 1 shows extremely intensive convection was observed in the southwestern part of Sri Lanka.

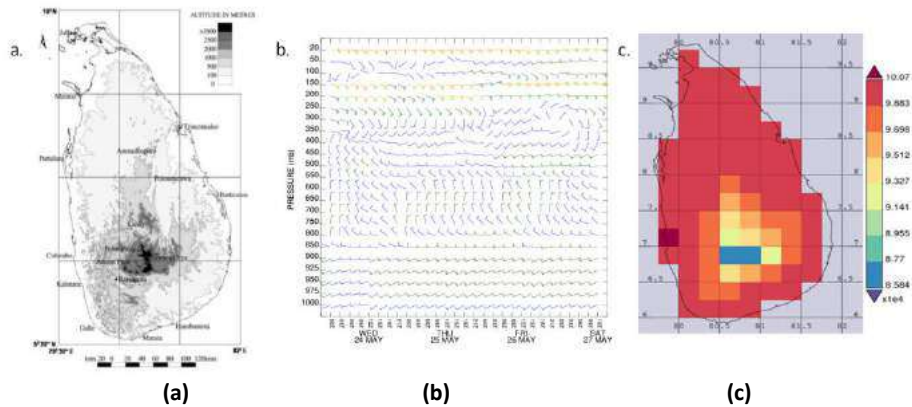


Figure 2. Image (a) Elevation map of Sri Lanka showing changing slope. Image (b) Wind gram over the south western part of the country at latitude 7.43 and longitude 80.74. Image (c) shows the time average map of surface air pressure (Pa) over Sri Lanka for May 2017

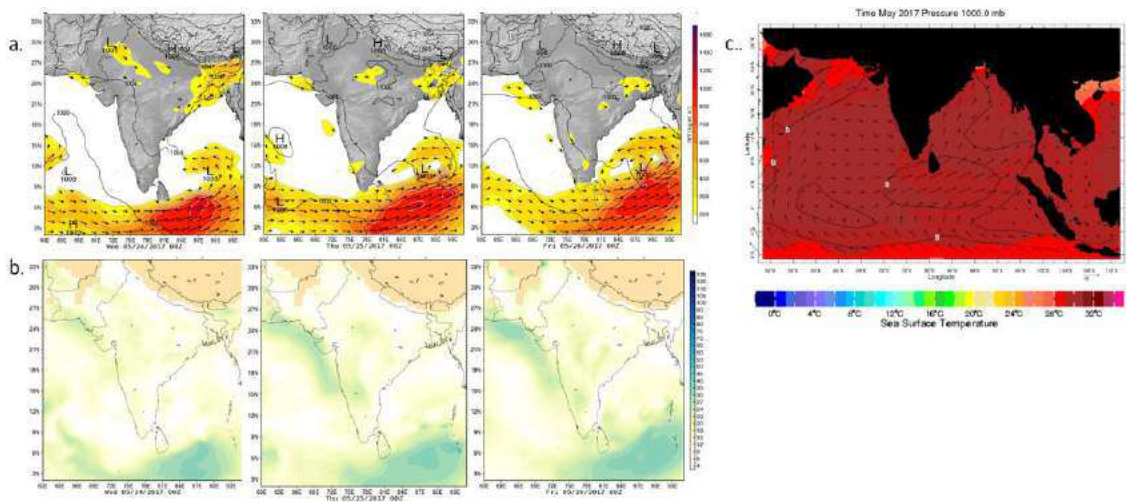


Figure 3. (a) Integrated water vapor transfer shows a Continuous supply of Moist air from 24 to 27 May in the Southwestern part of the country. (b) Prevailing easterly wind speed up to 10 ms⁻¹ at 850 hPa. (c) Horizontal wind analysis and tropical warm pool

Results and Discussion

The rainfall maps on 24th, 25th and 26th of May show heavy rainfall was confined over the Central and South-western parts of Sri Lanka which covers Sabragamuwa, South, West and Central provinces. The rainfall variation along the central highlands were show in figure 1. On 24th and 26th the strong convergent zones were detected in the windgram. A quasi-stationary extratropical low-pressure system that simulates the tropical low over the central high lands was produced. Figure 3 (a) shows that there was a confluent low-level flow was observed from the South-western part of the country to the Bay of Bengal. Over the Indian ocean counter-clockwise formation of deep depression was encountered at that period. This brings the cross-equatorial moisture flux over the southwestern part of Sri Lanka. Thus, a positive environment for the tropical storm surge was created, and consequently very heavy rainfall was observed in Southwestern Sri Lanka. Figure 3 (b) revealed a strong easterly wind at sea level with the maximum speed of 10 m s⁻¹ persisted over the west coast of Sri Lanka. Horizontal wind analysis at different levels shows figure 3 (c) that the wind from the East and West have converged around the South-west of Sri Lanka. This converging wind pattern induces surface upwelling of moisture over the North-eastern part of the Indian Ocean including Sri Lanka. Easterly winds were observed in the Southern part of Sri Lanka, which carries the moisture air parcels from the tropical warm pool to the East of Sri Lanka. Moist air uplifted by the slopes of the central high land mountain ranges induces orographic rainfall. The

repetitive formation of cells (figure 3 a) would simulate strong convection and propagate the upstream movement of convective cells along the slopes of the central hills. Hence strong orographic effect was simulated.

Conclusions and Recommendations

The study reveals the influence of the orographic effect on the extreme rainfall over Sri Lanka from 21st to 31st of May 2017. Localized heavy rainfall was initiated by the orographic lifting by the high moisture flow and steep orography of the island. This study investigates the effect of terrestrial orographic rainfall over Sri Lanka from the perspective of moisture exchange between ocean and land. Vertical acceleration of air parcel is favored by the physical processes such as moisture in the low level of the atmosphere, instability of air, and buoyancy processes. Therefore, an extreme convection was presented in the South-western part of the island. Yet orographic effect was principally account for flooding and the landslides. Moreover, the tropical depression intensified to tropical cyclone Mora on 28th of May 2017 over the Bay of Bengal.

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SHEAR STRENGTH CHARACTERISTICS OF UNSATURATED SRI LANKAN RESIDUAL SOILS AND ITS RELATIONSHIP WITH SOIL WATER CHARACTERISTIC CURVES

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Introduction

Rainfall induced slope failure is a major problem in most of tropical countries including Sri Lanka. It can be observed that during dry periods soil slopes can stand safely, on the other hand these fails during rainy periods. Even though there are several mitigatory actions are applied, year by year the problem of instabilities of slopes during rainy season becomes a significant news within the country.

Thus, as a sharp Early Warning System (EWS) is a needy of the country it is essential to understand the nature of unsaturated residual soil properties.

It needs advanced analyzes related to stability and infiltration [1] to estimate and evaluate the variation of shear strength parameters and unsaturated soil characteristics. To analyze those parameters, need to examine on Matric suction with Permeability and shear strength of unsaturated soils. The manifest way of determining these characteristics is developing a Soil Water Characteristics Curve (SWCC).

Hence, this research study had been done to obtain Soil Water Characteristic Curve (SWCC) and Permeability function using tensiometer method and shear strength parameters using direct shear tests. Further SWCC were developed for the soil using empirical models; Arya - Paris and Zapata.

Materials and Methods

After the sample collection from a landslide within Faculty of Engineering, University of Ruhuna – Disturbed samples, physical properties were found using basic tests. Then SWCC was developed using tensiometer method and was compared with two empirical models. In order to get continuous tensiometer readings and weight measurement, an Arduino setup was used.

Figure 1 shows the set up used to obtain SWCC. For drying curve, a fully saturated sample was allowed to dry in air until it become fully dried. For wetting curve, a fully dried sample was continuously wetted until it become fully saturated.

The base method used here was the method outlined by prof. Apiniti Jotisankasa [1].

Three tensiometers were connected to the sample on top, middle and bottom. From the readings of the tensiometers matric suction of top, middle and bottom was measured while mass change was obtained from electric balance.

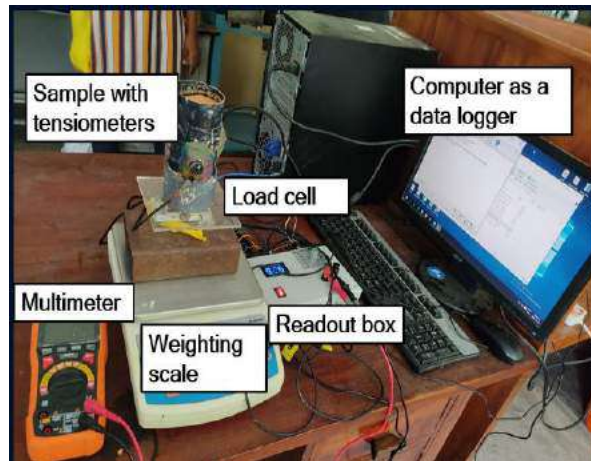


Figure 4. Setup used to obtain SWCC

Volumetric water content can be taken from the gravimetric moisture content using the following equation,

$$\theta_w = \omega \frac{\rho_d}{\rho_w} \quad (1)$$

Where,

ω = Gravimetric moisture content

ρ_d = Dry density of soil

ρ_w = Density of water

Kankanamge [2] has done same proceeded testings for two undisturbed and three disturbed samples. Following figure (Figure 2) shoes the SWCC obtained by Kankanamage.

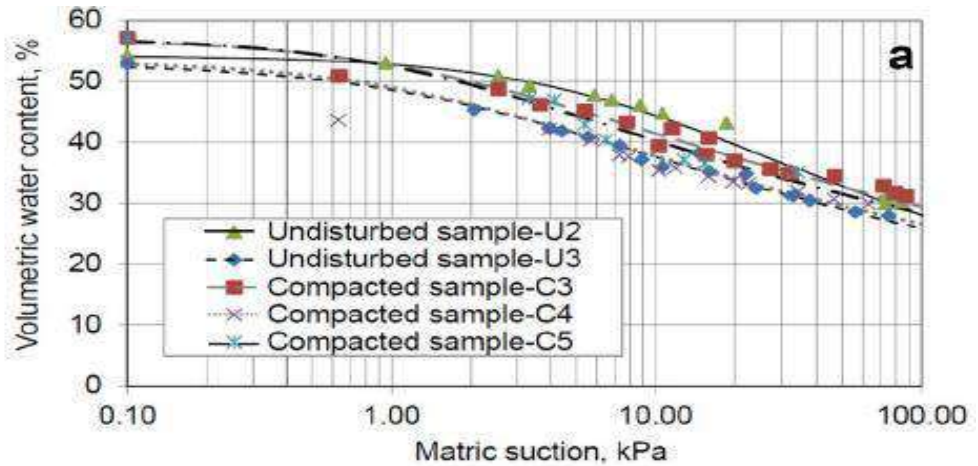


Figure 5. SWCC obtained by Kankanamge [2]

To obtain shear strength parameters series of direct shear tests were carried out using automated direct shear apparatus by changing saturation from 0% saturation to 100% saturation under six saturation levels of an equal mass of soil. Suction of each soil sample was measured at the end of each test and relationship between matric suction and shear strength was developed. Each of sample was loaded with three Normal load cases, 12.73 kN/m², 25.46 kN/m², and 38.197 kN/m² - under drained conditions with a shearing rate of 0.125 mm/min [3].

Permeability function was developed using the data collected from SWCC tests by incorporating them with following equations from literature.

$$i = \frac{d(z - \frac{s}{\gamma_w})}{dz} \quad (2)$$

Where,

z - elevation head of each tensiometer relative to the base of sample

s - matric suction,

γ_w - unit weight of water

$$v = \frac{dV_w}{A dt} \quad (3)$$

Where,

dV_w - change of volume of water in soil sample (can be calculated by change in soil mass)

A - cross section area of sample

dt - elapsed time.

The value of permeability at any suction and volumetric water content can then be calculated by,

$$k = v / i \quad (4)$$

k- Hydraulic conductivity

Jotisankasa [1] and Kankanamge [2] had made relationships between matric suction and shear strength using several methods, but all graphs showed non linear relationships with different regration values.

Results and Discussion

Basic soil properties

From the Sieve analysis test particle size distribution was obtained as 71% passing from 0.075mm sieve. With the help of basic property tests, according to Unified Soil Classification System (USCS), soil was classified as CL- Inorganic clay of low to medium plasticity.

Table 1 shows the summery of the basic physical properties.

Table 2. Summary of physical properties

Test	Parameter	Value
Saturation test	Fully saturated moisture content	70%
Specific gravity test	Specific gravity (GS)	2.602
Hydrometer analysis	Clay content (0.002mm passing)	30%
Atterberg limit test	Liquid Limit	49%
	Plastic limit	28%
	Optimum moisture content	21%
Proctor compaction test	Maximum dry density (kN/m ³)	14.75

SWCC test results

Figure 3 shows the obtained SWCC from the tensiometer method (wetting and drying) and from the empirical methods Arya- Paris and Zapata. For the comparison for both drying and wetting paths, curves related to middle suction data are considered here; as those two curves show lesser scattering.

For both wetting and drying curves shape of the SWCC came more relatable with the shapes of literature (Figure 2), but small scattering nature was obtained and can be related with the sensitivity of the Arduino set up.

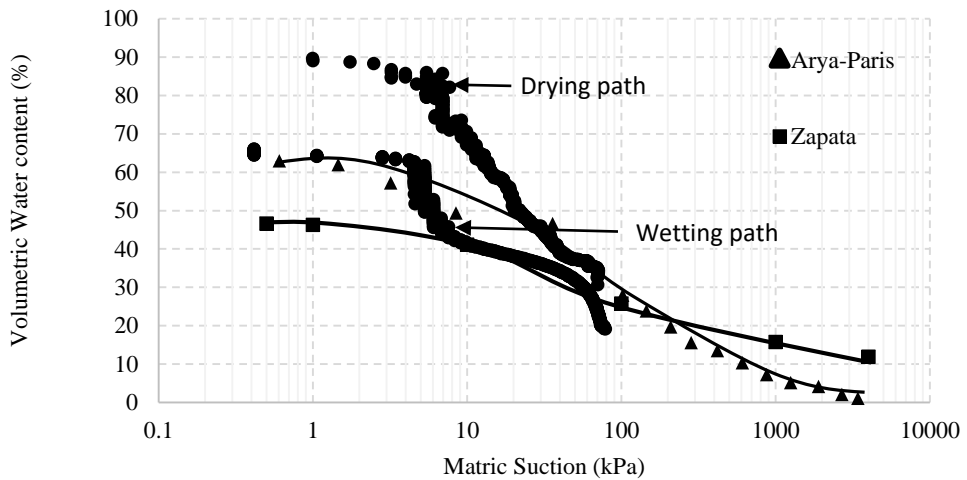


Figure 6. SWCC developed for the soil

Table 2 shows the important points found from obtained SWCC. But it showed that experimental curves and modelled curves are not completely tallying. These two empirical methods were related to numerical equations and as the input parameters only particle size gradation and plasticity indexes were needed. Therefore, these can relate to a range of that particular type of soil.

Table 3. Important points of SWCC

Curve	Air Entry Point		Inflection point		Residual water content	
	Volumetric water content (%)	Suction (kPa)	Volumetric water content (%)	Suction (kPa)	Volumetric water content (%)	Suction (kPa)
Arya and Paris	60	4.01	60	4.01	3.5	1200
Zapata	45	3.9	45	3.9	-	-
Wetting path	65	4.2	65	4.2	-	-
Drying path	86	4.2	86	4.2	-	-

Direct shear tests

Figure 4 shows the variation of shear strength parameters; they increase and reach maximum values at optimum moisture contents (where it has the best particle arrangement) and then gradually decrease with the increase of saturation

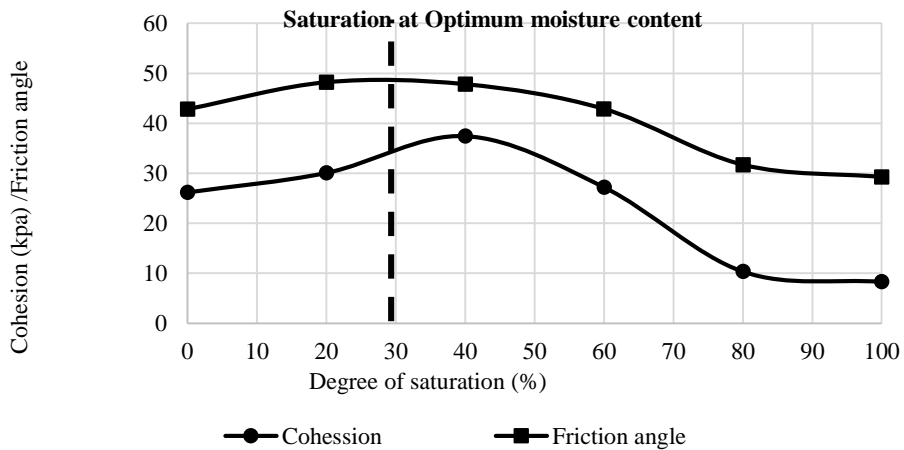


Figure 7. Variation of shear strength parameters

When the soil is fully dry, the compaction it can achieve is less hence lesser strength. But with the increase of moisture up to optimum moisture content, particles will well packed and will achieve its maximum shear strength.

Then further increase of the moisture from the optimum value will cause the flocculated structure to become dispersed structure causing loss of matric suction hence loss of its shear strength.

Figure 5 shows the relationship of cohesion and matric suction where cohesion increase with matric suction in a nonlinear way; where similar kind of results obtained by Jotisankasa [1] and Kankanamge [2].

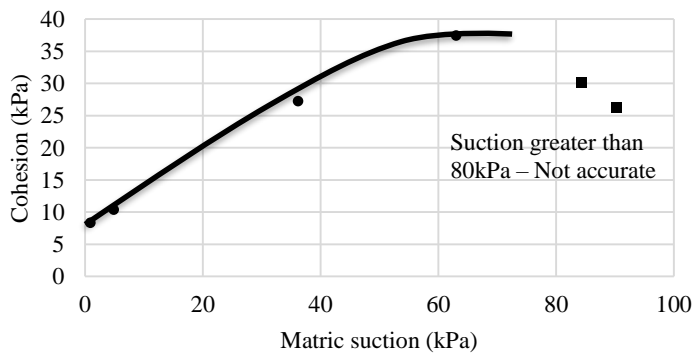


Figure 8. Relationship of cohesion and matric suction

Permeability function

Figure 6 shows the permeability function obtained from middle tensiometer data.

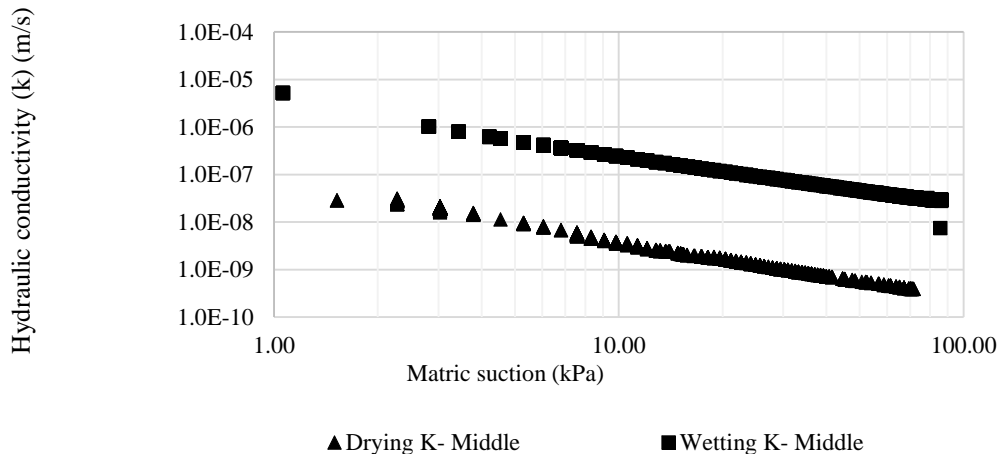


Figure 9. Permeability function

Permeability of the soil increase as it gets saturated gradually due to filling of the voids inside it. Therefore, at the beginning of wetting soil has lower permeability – water has plenty of voids to fill. When time passes and if wetting continued permeability will increase with the time.

The hydraulic conductivity decreases with increase of suction.

Conclusions and Recommendations

Cohesion and friction angle of soil decrease with the increase of saturation depicting that slope fail in wet season; shear strength of soil reduces as it gets more and more saturated but decreased after it reached to its saturation at optimum moisture content.

Obtained SWCCs from experimental data can be used as base data for an early warning system. Further analyzing these base data as hydraulic conductivity function and porewater pressure variatinn functions are present, can find the noiminal rainfall intencity for a marginal slope stability. From the permeability function, permeability increases at lower suctions and decrease when suction increases. Here it can be concluded that water infiltrate more with the increase of saturation of the soil. As these experiments are done for a single slope failure, more tests should be carried out for different slope failures and not only for failed slopes but also for residual soil types in different locations of Sri Lanka

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IDENTIFICATION OF BASIC PROPERTIES OF MUNICIPAL SOLID WASTE IN MEETHOTAMULLA OPEN DUMP SITE

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Introduction

Disposal of Municipal Solid Waste (MSW) has become a serious problem in all over the world, which is affected by the rising population and rapid urbanization. Most of the developed countries trend to proceed with engineered landfilling or incineration of solid waste while the developing countries continue with open dumping. Due to absence of advanced technologies and a higher amount of cost requirement, open dumping is the most popular method of waste disposal in developing countries like Sri Lanka. However, due to the lack of horizontal land space and higher demand for prime lands in these city areas, vertical expansion is the solid waste management method adopted in these open dump sites. Due to the decomposition of biodegradable solid waste, settlement would be induced during the operational stage of the dump sites which is directly affected by the stability of the garbage mounds. Maintenance of proper slope during the operational stage is important to prevent occurrence of slope failures which may directly affect on workers who are directly involved in operation, neighbors who are living around the dump sites and several infrastructures near the garbage mounds. After the closure of these dump sites, the land areas would be reused for industrial zones, recreational purposes (eg; Golf ground) or land reclamation activities. Therefore, obtaining the accurate basic properties of MSW with aging is timely important under the two scenarios. As such findings of this research would give great assistance to achieve geophysical, chemical and compaction characteristics of MSW under the two scenarios.

Materials and Methods

In order to determine the physical, chemical and compaction characteristics of MSW, considering the aging effect, following procedure was carried out.

Collection and preparation of samples

Waste samples were extracted from the Meethotamulla dump site which is located in Kolonnawa area during the restoration phase. Four number of disturbed waste samples were collected at 4 m, 8 m and 16 m levels from Meethotamulla dump site using a bache. The four different depths represent the aging effect of MSW. After air-drying the extracted samples, they were laid on the dry concrete surface and large debris were subdivided into small parts. It

should be reduced the samples according to the SATREPS, 2014 testing manual. The procedure of reducing the samples was carried out according to the quartering method. 5-10kg of samples were taken to the laboratory experiments.

Physical properties of MSW

01. Waste composition

By referring to a standard manual called JIS A 1204 and SATREPS Project manual, the composition of MSW were determined for each sample which represents different ageing. According to the SATREPS manual, MSW can be divided into 13 main categories such as Kitchen waste, Paper, Hard plastic, soft plastic, Metal, Glass, Ceramic, Leather and Rubber, Textile, Grass and Wood, Rock, cemented material, Aggregated soil and others. After sorting the MSW into above stated categories by visual observations, weight fractions were calculated to find the composition of each category.

02. Particle size distribution

After referring JIS A 1204, ASTM D422 and SATREPS Manual, Sieve Analysis test was conducted for the whole waste sample. The entire air-dried waste samples were sieved using 63, 37.5, 19, 9.5, 4.75, 1.7mm and 0.075mm diameter sieves and passing percentages were determined.

03. Specific gravity

In order to determine the particle density of MSW, Specific gravity test was conducted for the large-size particles and small-size particles separately. Large-size particles consisted of particles with 2mm to 10mm and small-size particles consisted of particles having less than 2mm diameter according to the SATREPS manual.

04. Bulk unit weight

According to the SATREPS manual, bulk unit weight of MSW was determined. Prepared waste sample was put completely into a container which has known volume and container was fallen down to the ground from 30cm height for 3 times. Then it should be placed some additional waste into the container and repeated the above activity.

05. Saturated moisture content

Saturated moisture content is the maximum water content that can be stored in the waste sample. In order to perform this test, the waste samples were sunk in water, after taking the initial moisture content. Then moisture content was checked every day until it will become a constant value. When the moisture content was not changed, this value was considered as the saturated moisture content.

Chemical properties of MSW

01. pH value

pH of the MSW was measured using glass- electrode pH meter at several temperature and test method for determining pH, is mentioned in the JGS 0211. Firstly, air dried waste samples were taken for testing. Then particles larger than 10mm were removed from the samples by sieving. The amount of sample for the measurement was selected according to the maximum particle size of the sample. Then, raw samples were placed in the beakers and distilled water was added. The mass of water in the beaker should be five times the dry mass of the sample. Finally, the samples were stirred using a glass rod and kept it for 30-180 minutes. By using Glass- electrode pH meter, pH values were measured.

02. Electric conductivity (EC)

Test method for determining EC, is mentioned in the JGS 0212 and this was measured using Electrical conductivity sensors. Sample preparation method for EC is same as the pH.

03. Volatile organic content

Volatile Organic Content of MSW was measured according to the JIS A 1226. This is an important parameter which can be affected directly towards the stability of dump site because organic materials can be decomposed easily, within a short time period. The oven dried waste samples at 60°C were again dried up to 800°C in the Muffle furnace to remove the volatile organic components of the MSW.

Compaction characteristics of MSW

In the field, compaction of waste is done using rollers or dynamic compactors and this is simulated in the laboratories by standard Proctor compaction test. To develop the compaction curve, automated Proctor compaction apparatus was used. Compaction is a type of densification of soil or waste by removing the air from voids. Higher Compaction leads to improve the density and strength characteristic of solid waste while decrease the compressibility and permeability characteristics. Standard Proctor Compaction Test was performed according to ASTM D 698, which is equivalent to JIS A1210.

Results and Discussion

After performing the laboratory experiments, physical, chemical and compaction characteristics of MSW were determined considering the aging effect. The obtained results were analysed as below.

Physical and chemical properties of MSW

01. Waste composition

It can be observed that the largest portion of the waste materials is consisted with Aggregate soil, Polythene and Fine residues. The large portion of having fine residues and aggregate soil is due to the partially decomposed waste from the site. Meethotamulla bottom waste consisted with more fine residues than the other samples and percentage of fine residues is increasing with the depth. The

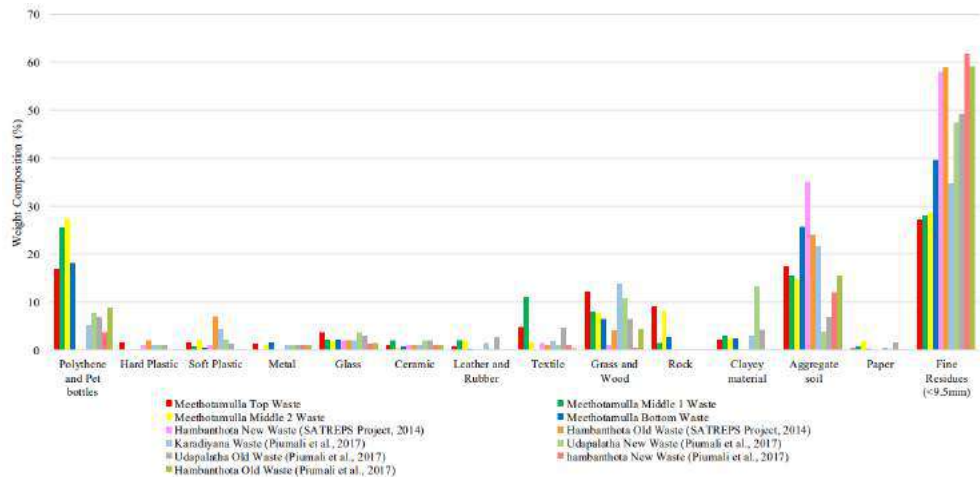


Figure 1. Comparison of composition of MSW in different dump sites in Sri Lanka

According to the Figure 1, the largest portion of the waste materials are consisted with fine residues and aggregate soil. In most of the garbage dumping yards, cover soil layers are applied on the waste layer to prevent the animal attraction, bad odor and displeasing view of the garbage mound. Then cover soil is easily mixed with the waste materials, when extracting waste from the several depths. Further, due to the decomposition of organic waste materials, fine particles can be accumulated at the bottom of the garbage mound and these are the possible reasons for having the large amount of aggregate soil and fine residues.

02. Particle size distribution

In order to compare the particle size distribution of MSW in other dump sites, data published by Balasooriya et al., 2015 and Nawagamuwa et al., 2013 were used. Comparison of particle size distribution of MSW in present study is compared with the existing data as shown in Figure 2.

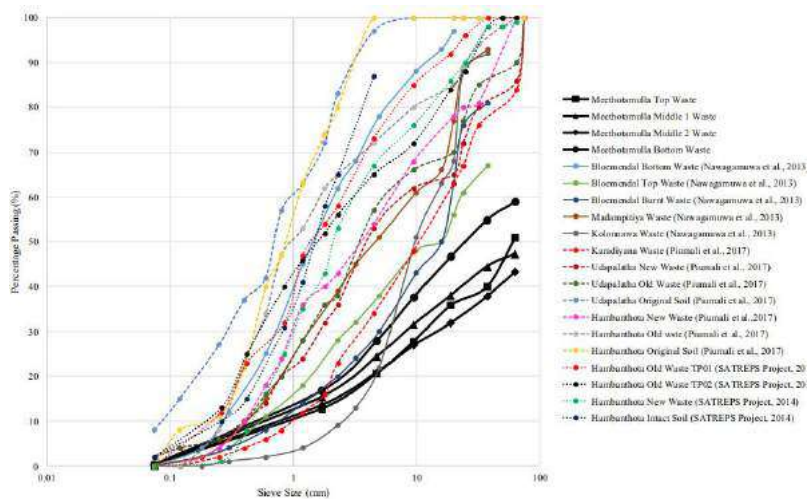


Figure 2. Comparison of particle size distribution of different dump sites in Sri Lanka

The particle size distribution curves obtained for the Meethotamulla garbage dump are in the range of particle size distribution behavior of Bloemendhal waste dump and the Kolonnawa waste dump [1]. Further these curves are deviated from the Hambanthota waste [2] and Udapalatha waste [3]. Climatic differences of different regions would be a reason for this deviation because Meethotamulla, Karadiyana and Bloemendhal waste dump sites are in Colombo region which belongs to the wet zone of Sri Lanka while Hambantota belongs to the dry zone of Sri Lanka. Climatic condition of the area is affected to the decomposition rate of garbage because microorganisms are very sensitive with the temperature and humidity of the surrounding environment.

It can be observed that more than 40% of the particle sizes are greater than 63mm and it includes most of the elongated, large debris such as polythene, pet bottles, hard plastics, metal, glass, ceramic, leather, textile, yard waste, wood, clayey materials and rock etc. Summary of physical and chemical properties of MSW at different depths is shown in Table 1.

Table1. Physical and Chemical Properties of MSW of Meethotamulla dump site with aging

Sample	Bulk Unit Weight (kN/m ³)	Gs (Large particles)	Gs (Small particles)	pH	EC Value (mS/cm)	Volatile Organic Content (%)
Top Waste	6.22	2.16	2.10	7.54	2.96	39.1
Middle Waste 1 (@4m)	4.78	2.20	2.16	7.60	2.03	39.0
Middle Waste 2 (@8m)	4.28	2.24	2.21	7.39	1.81	38.8
Bottom Waste (@16m)	8.19	2.25	2.23	7.61	1.17	38.7

Compaction characteristics of MSW

Based on the results, it can be noted that the maximum dry unit weight is 11.2kN/m³ and the optimum moisture content is 38%. The variation of dry unit weight over moisture content is illustrated in Figure 3.

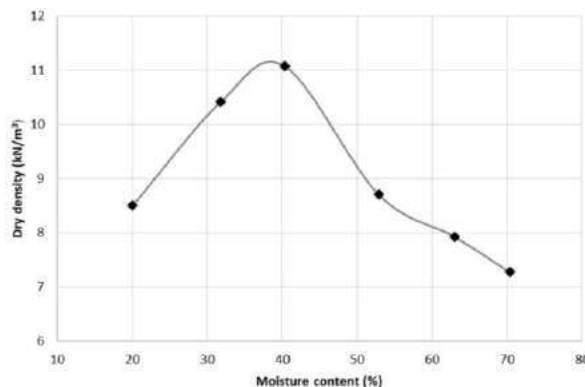


Figure 3. Proctor Compaction Curve

Conclusions and Recommendations

Specific gravity values are high due to the large composition of fine residues and they are increasing with aging. pH values are greater than 7.0 because waste was fully decomposed. Electric conductivity of top waste is high due to the presence of metals and they are decreasing with aging. There is a considerable amount of fabric composition due to the rapid development of textile industry.

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ANALYSIS OF ALKB2 AND LCA^R OPERON GENE EXPRESSION IN RESPONSE TO N- ALKANES IN *Pseudomonas aeruginosa* PAO1

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Introduction

Crude oil is a mixture of hydrocarbons. They are not soluble in water. Thus, hydrocarbon contamination in soil, and water greatly reduces the bioavailability of nutrients to the bacteria. The capability of hydrocarbon utilization provides competitive advantages to microorganisms as they are provided with carbon and energy to persist in the hydrocarbon contaminated sites [1]. The major constituent of crude oil is n-alkanes. *Pseudomonas aeruginosa* is the most common ubiquitous, gram-negative, aerobic, and free-living bacterium that can degrade varying lengthy n-alkanes as a source of carbon and energy [2]. It encodes two alkane-1-monooxygenases (AlkB1 and AlkB2) and these enzymes are involved in a pivotal role in the alkane degradation pathway which is the catalysis of conversion of n-alkanes into n-alkanols [3]. However, the mechanism of n-alkane utilization of *P. aeruginosa* PAO1 is still under investigation. We have previously reported that a GntR family regulator that transcribes divergently to alkB2 gene is responding to n-alkanals and it specifically interacts with promoter region of alkB2 and lca^R operon [4,5]. The objective of this study is to determine *in vivo* differential gene expression of alkB2 and lca^R operon in response to exogenous different carbon lengthy n-alkanes to fill the important knowledge gap required for understanding the mechanism of n-alkane degradation pathway of the *P. aeruginosa* PAO1.

Materials and methods

Growth of P. aeruginosa on glucose and different lengthy n-alkanes

P. aeruginosa cells were harvested from an overnight culture grown in M9 minimal media supplement with glucose as a source of carbon and washed with M9 media free-from glucose to remove residual glucose. The M9 media (10 ml) was supplemented with 0.2% (v/v) or (w/v) n-tridecane, n-hexadecane, n-eicosane, n-docosane, and n-octacosane separately, were inoculated with 20 µl of *P. aeruginosa* cells diluted to an OD 0.01 and incubated at 37 °C. The effects of the volume reduction, pigment production, and biofilm formation on the measurement of OD were avoided. The total cells were harvested from three flasks per day for each alkane and each cell pellet was completely dissolved in 10 ml of M9 media free-from alkanes and the OD was measured. Growth curves were drawn after seven days by plotting the OD vs time.

Total RNA isolation from P. aeruginosa grown on different carbon sources

P. aeruginosa cells were harvested from an overnight culture grown in M9 minimal media supplement with glucose (source of carbon) and washed with M9 media free-from glucose to remove residual glucose. Then M9 medium (50 mL) supplement with 0.2% (v/v and w/v) glucose, n-tridecane, n-hexadecane, n-eicosane, n-docosane, and n-octacosane separately, were inoculated with 20 µl of *P. aeruginosa* cells diluted to an OD 0.01 and incubated at 37 °C for few days until it reached the OD 0.1- 0.2. Next, total RNA was isolated using Promega total RNA isolation kit (Cat. Z3100) according to the instructions given by the manufacturer.

cDNA synthesis

The cDNA synthesis was done in the Labnet MultiGene OptiMax Thermal Cycler (Model TC9610). Firstly, cDNAs were synthesized separately for *16s rRNA* gene, *alkB2*, and *lacR* operon by using 1 µl of each 20 µM primer (Table 1), 700 ng of total RNA, and nuclease free H₂O up to 15 µl. Reaction mixtures were incubated for 5 min at 70 °C. Just as the incubation ends, the vials were transferred to an ice bath and were incubated for another 5 min in ice. Next, M-MLV 5x reaction buffer (5 µl), 10 mM dNTPs (5 µl), and 40 000U M- MLV reverse transcriptase (0.5 µl) were added to each reaction. All the vials were incubated at 49 °C for 60 minutes.

Table1. Primers: cDNA synthesis

Primer	Sequence	T _m of the melt curve (50 mM NaCl)
alkB2 RT	5'- TCA GAT GCG CTG GGT GTC GGT AAG C -3'	64.8°C
PA1527 RT	5' - GAATGGATATCTCGGCGTACTG - 3'	54.9°C
Control RT	5'- GGA GGT GAT CCA GCC GCA G-3'	60.9°C

Quantitative real time PCR

The quantitative real time PCR was performed in the CFX96 touch real-time detection system, Bio-Rad in 10 µl reactions. The genes, *alkB2*, *lcaR*, *PA1526*, and internal control *16s rRNA* were amplified with 0.5 µl of 10 µM sequence specific primers (Table 2) and 2 µl of cDNA using Qiagen qRT PCR kit (Cat: 208054). SYBR green I fluorescence is the amplification reporter. qRT-PCR was performed in triplicate and validations had been carried out before the comparative C_t (2^{-ΔΔC_t}) method was applied for data analysis.

Table2. Primers: qRT PCR primers

Primer	Sequence	T _m of the melt curve (50 mM NaCl)
alkB2_fwd	5' - AACTGCCCAACGGCTATG - 3'	55.6°C
alkB2_rv	5' - GGTGTCGGTAAGCTGGTATTC - 3'	55.3°C
lcaR_fwd	5' - GATCGTTCAGCTCTACATCCTG - 3'	54.9°C
lcaR_rv	5' - AGCCGTCGATATCGCTTTG - 3'	55.2°C
PA1527_fwd	5' - ATCTTCAACGGCTCGAATACC - 3'	54.8°C
PA1527_rv	5' - GAATGGATATCTCGGCGTACTG - 3'	54.9°C
Control_fwd	5' - ACTGAGCTAGAGTACGGTAGAG - 3'	54.7°C
Control_rv	5' - CCACTGGTGTTCCTTCTATATC - 3'	54.6°C

*fwd indicates forward primer and rv indicates reverse primer of a particular gene

Results and Discussion

Growth of P. aeruginosa PAO1 on different lengthy n-alkanes

The growth of *P. aeruginosa* PAO1 in M9 minimal media supplemented with different lengths of n-alkanes (carbon source) was monitored and was compared with growth on glucose. n-Hexane, n-tridecane, n-hexadecane, n-eicosane, n-dodecane, n-octacosane, and glucose were used and growth curves were drawn using cell density vs time. *P. aeruginosa* grown on glucose was reached the stationary phase after 24 h (Figure. 1A) and no growth was observed on n-hexane (Figure. 1B). Cultures grown on n-hexadecane, n-eicosane, n-dodecane, n-octacosane reached the stationary phase within seven days (Figure. 1D, 1E, 1F, and 1G) while 14 days on n-tridecane (Figure. 1C) indicating *P. aeruginosa* PAO1 can degrade n-alkanes rapidly as the carbon chain length is increased. However, these results indicate that among all carbon sources, *P. aeruginosa* PAO1 efficiently utilizes glucose than any of the n-alkanes. Alternatively, no growth on hexane revealed *P. aeruginosa* PAO1 cannot utilize small chain alkanes as a source of carbon and energy. In this study, we found that *P. aeruginosa* PAO1 can utilize n- alkanes ranging from C13 to C28 (Figure. 1).

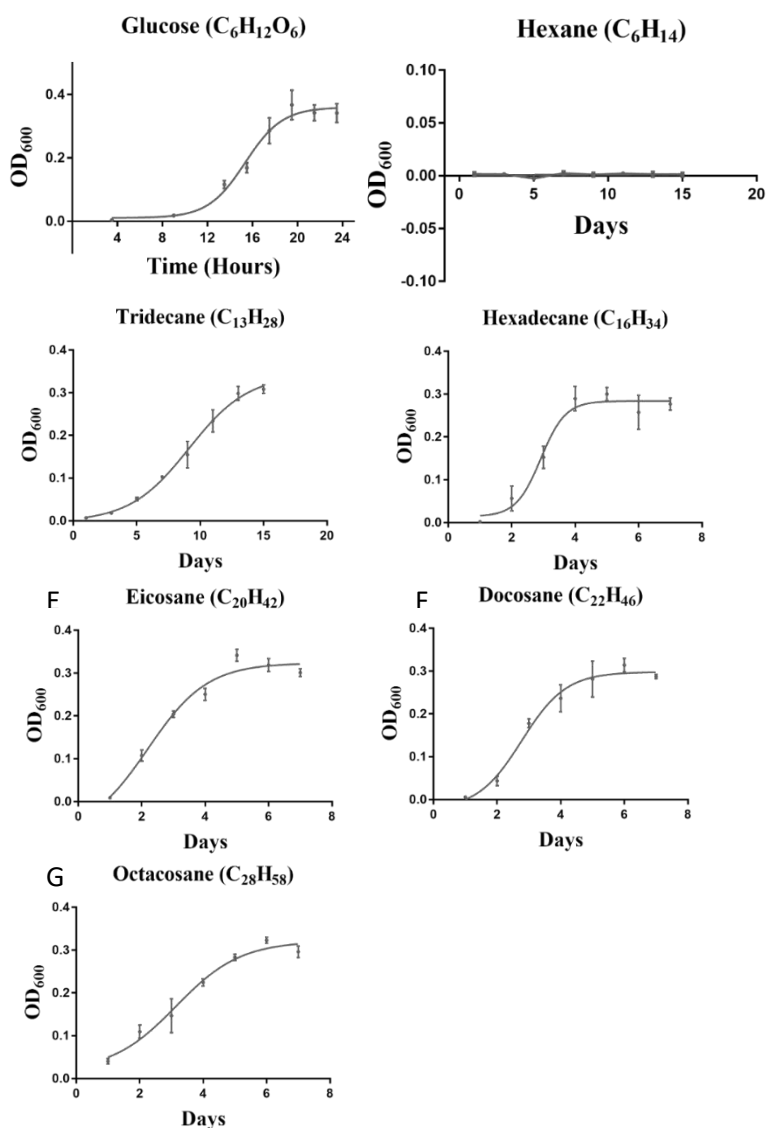


Figure 10. Growth of *P. aeruginosa* PAO1 on different carbon sources. A). Glucose, B). Hexane, C) Tridecane, D). Hexadecane, E) Eicosane, F) Docosane, and G) Octacosane

Gene expression analysis

To articulate the contribution of *lcaR* for the utilization of n-alkanes, the expression profiles of the genes; *alkB2*, *lcaR*, *PA1527*, in the presence of different lengths of n-alkanes were analyzed using glucose as the control. The expression profile indicates that all three genes are up-regulated in higher folds in the presence of tridecane and hexadecane compared to the other n-alkanes. The greatest expression of *alkB2* occurs in hexadecane (fold change 34), next in tridecane (Fold change 27) while the other genes remain up-regulated with equal

folds (fold change of *lcaR* is about 28 and fold change of *PA1527* is about 12). However, a low level of *alkB2*, *lcaR*, and *PA1527* expressions were observed in eicosane and docosane. The fold changes were 4, and 6 respectively. In octacosane, *alkB2* is slightly expressed with a 1.7-fold change while the other two genes were down-regulated (Figure. 2). The n-alkane oxidation pathway of medium chain alkanes was initiated with the terminal oxidation of alkyl group by *AlkB2* and proceeds via alkanol, alkanal, and alkanolic acids to medium chain acyl CoA. The nature of the divergent promoter and transcription start site may be the reason for the difference in fold change of expression levels of *alkB2*, *lcaR* and *PA1527*. The utilization of long chain alkanes as a source of carbon might also be mediated by other enzymes such as *AlkB1*. The small up-regulation of *alkB2* and *lcaR* operon when long-chain alkanes (C20 – C28) are available might be caused by the production of medium chain n-alkanes or their oxidative derivatives through the long chain n-alkane degradation pathway or leaky expression.

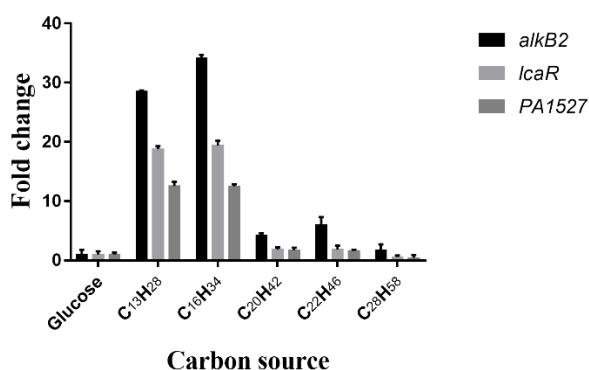


Figure 11. The *alkB2*, *lcaR*, and *PA1527* expression on different carbon sources

Conclusions and Recommendations

When compared to the growth curves and the gene expression profile with our previous findings, the link between *alkB2* and *lcaR* was identified in such a manner that *LcaR* acts as a repressor for the *alkB2* gene and *lcaR* operon. This is because when there are no medium chain alkanes available, *LcaR* binds specifically to the promoter region (*lcaO*). Thereby transcription of the *alkB2* gene and *lcaR* operon is hindered. When the medium chain n-alkanes are available, the alkane oxidation pathway activates owing to leaky expression of *alkB2* gene, and medium chain alkanals are produced. Medium chain alkanals act as the signaling molecules of *LcaR* altering the DNA binding conformation upon binding to *LcaR* so that they no longer can repress the *alkB2* and *lcaR* operon expression. The gene expression analysis clearly shows, *alkB2* and *lcaR* responded well only if the growth medium contains medium-chain n-alkanes, though cell densities are increased as the carbon chain length is increased in n-alkanes.

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FOCUS AREA
Food, Nutrition and Agriculture

INVESTIGATION OF EAR MORPHOLOGY AND ITS RELATIONSHIP WITH GROWTH PARAMETERS IN LOCAL GOATS

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Introduction

Goat rearing is popular among rural farmers since the investment in resources is relatively low. More than 70 % of the population of Sri Lanka is engaged in rural-based agriculture. High adaptability, high productivity and non-competitive with feed are beneficial for goat farmers [1]. Goats are well adapted to different environmental conditions and feed resources [2]. The climatic conditions of Sri Lanka is more conducive to goat farming, and it has the potential to grow the country's economy. In Sri Lanka, 75 % of goats are reared in dry zones and intermediate zones. The optimum climate conditions can be obtained for goat farming from dry zones [2].

Despite the vast resources of goats in the country, their productivity is less than expected. One of the reasons for this is the selection of breeds with low productivity [3]. The use of morphological characterization can be more critical for farmers to determine the quality of goat due to its relatively low cost and high accuracy [4]. Several goat breeds are reared in Sri Lanka, and the morphological characteristics vary according to breeds. Understanding the relationship between morphological characteristics and growth parameters of local goat breeds is essential, especially when selecting goats for rearing for meat purposes [5]. Moreover, identification of variation in morphological traits is the essential step toward characterization of livestock breeds. Therefore, the present study was conducted to investigate ear morphology and its relationship with growth parameters in local goats reared in Ampara district.

Materials and Methods

A. Location

The study was conducted in the selected five veterinary ranges (Alayadiwembu, Addalachennai, Kalmunai - MD, Pothuvil, Thirukkivil, Uhana) in Ampara District, a part of the low country, which is located in the dry zone. The climate is generally tropical, with annual temperatures ranging from 24.4 to 31.7 degrees Celsius.

B. Data collection

The 30 randomly selected goat samples were obtained from the selected five veterinary ranges, of which 15 were female goats and 15 were male goats. Data were collected using a pre-tested structured questionnaire which had the following information: Qualitative parameters (ear orientation, ear shape), and quantitative parameters such as Bodyweight (BW), Body Length (BL), Height at Withers (HW), Chest Girth (CG), Chest Width (CW), Ear Length (EL), Ear Width (EW), Tail Length (TL), Horn Length (HL), Rump Length (RL), Pelvic Width (PW), Length of Head (LH). The weight of goats was measured and recorded individually using a 100 kg weight scale to estimate live body weight. Body measurements were taken using a measuring tape (cm). The linear body measurements were taken while the animals were standing on a flat surface.

C. Data analysis

The recorded data were entered in the MS Excel 2016 spread sheet and imported to IBM SPSS statistics 25 for data analysis. Simple descriptive statistics were used for qualitative parameters, and the Correlation coefficient was used for quantitative data analysis.

Results and Discussion





A. Qualitative variable

The horizontally carried ear was the most common ear orientation (47%) among the goat breeds evaluated. According to the final analysed data, 30% was pendulous, and 23% was semi-pendulous. Considering the shape of the ears, 57% of the goat had straight-edged ears, 23% had semi-straight ears, and 20% had curly ears reported (Table 1a, 1b).

Table 1a. Goats ear morphology description

Variables	Percentage
Ear Orientation	
Pendulous	30%
Semi-pendulous	23%
Carried horizontally	47%
Ear shape	
Straight-edged	57%
Semi-straight	23%
Curly	20%

Table 4b. Goats ear morphology schematic diagram

Ear orientation		Ear shape	
Pendulous		Straight-edged	
Semi-pendulous		Semi-straight	
Carried horizontally		Curly	

B. Quantitative variables

Table 2a. Correlation between ear morphology and growth parameters of female goats

	BW (kg)	BL (cm)	HW (cm)	CG (cm)	CW (cm)	EL (cm)	EW (cm)	TL (cm)	HL (cm)	RL (cm)	PW (cm)	LH (cm)
BW (kg)	1											
BL (cm)	.777**	1										
HW (cm)	.838**	.864**	1									
CG (cm)	.846**	.871**	.914**	1								
CW (cm)	.637**	.439	.577**	.642**	1							
EL (cm)	.328	.682**	.628**	.645**	.070	1						
EW (cm)	.324	.665**	.559*	.646**	.029	.960**	1					
TL (cm)	.446*	.627**	.502*	.375	.312	.239	.159	1				
HL (cm)	.319	.061	.309	.168	.250	-.365	-.299	-.052	1			
RL (cm)	.761**	.929**	.897**	.872**	.374	.803**	.770**	.566**	-.077	1		
PW (cm)	.718**	.612**	.743**	.752**	.715**	.334	.340	.426	.435	.635**	1	
LH (cm)	.729**	.890**	.893**	.898**	.507*	.750**	.710**	.409	-.017	.908**	.602**	1

** . Correlation is significant at the 0.01 level (2-tailed) *. Correlation is significant at the 0.05 level (2-tailed)
Bodyweight (BW), Body Length (BL), Height at Withers (HW), Chest Girth (CG), Chest Width (CW), Ear Length (EL), Ear Width (EW), Tail Length (TL), Horn Length (HL), Rump Length (RL), Pelvic Width (PW), Length of Head (LH).

The correlation analysis was used to analyse the relationship between the dependent variable(s) and independent variable(s). Ear morphological parameters (EL, EW) and growth parameters (BL, HW, CG, RL, LH, PW) had a positive correlation and statistically significance at 0.05 in female goats. Among these, a moderate positive relationship was observed between EL and EW and BL, HW, CG, RL, and PW in male and female goats (Table 2a, 2b).

Conclusions and Recommendations

Identifying the morphological characteristics of goats is important for identify animals with superior traits and for raising animals for meat. Perhaps the morphology of the goat's ear has no effects on their weight. Carried horizontal ear orientation and straight-edged ear shape are more common qualitative ear morphological parameters. The correlation analysis indicated that a moderate positive relationship was observed between EL and EW and BL, HW, CG, RL and PW in male and female goats. Further, more detailed research studies with large number of samples are needed.

Table 2b. Correlation between ear morphology and growth parameters of male goats

	BW (kg)	BL (cm)	HW (cm)	CG (cm)	CW (cm)	EL (cm)	EW (cm)	TL (cm)	HL (cm)	RL (cm)	PW (cm)	LH (cm)
BW (kg)	1											
BL (cm)	.853**	1										
HW (cm)	.867**	.915**	1									
CG (cm)	.873**	.892**	.925**	1								
CW (cm)	.801*	.891**	.933**	.959**	1							
EL (cm)	-.061	-.152	.097	.021	-.027	1						
EW (cm)	.257	.294	.496	.302	.304	.749*	1					
TL (cm)	-.256	-.078	-.282	-.106	-.143	-.053	-.494	1				
HL (cm)	.714	.782	.781	.936**	.752	.546	.608	.312	1			
RL (cm)	.817*	.832*	.745*	.837**	.709*	-.096	.303	-.195	.616	1		
PW (cm)	.675	.726*	.768*	.764*	.852**	.099	.474	-.378	.749	.592	1	
LH (cm)	.892**	.892**	.976**	.904**	.892**	.069	.534	-.439	.708	.811*	.792*	1

** . Correlation is significant at the 0.01 level (2-tailed) * . Correlation is significant at the 0.05 level (2-tailed)
 Bodyweight (BW), Body Length (BL), Height at Withers (HW), Chest Girth (CG), Chest Width (CW), Ear Length (EL), Ear Width (EW), Tail Length (TL), Horn Length (HL), Rump Length (RL), Pelvic Width (PW), Length of Head (LH).

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RATE OF INFLORESCENCE EMMITTANCE REVEALS PROSPECTS FOR INTER-SPADIX SELF-POLLINATION IN SRI LANKAN TALL COCONUT (*Cocos nucifera* L.)

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Introduction

Coconut (*Cocos nucifera* L.) is the most extensively grown and used palm in the world. Depending on the stature and the breeding behaviour of the plant, coconuts are globally classified in to two varieties as Talls “*Typica*” and Dwarfs “*Nana*”. Among the two main varieties of coconut, the tall variety is important as the main variety of commercial plantations and as parent material in hybrid coconut seednut production. The planting material of tall cultivars are obtained through mass selection and open pollination. Even though coconut is a monoecious crop, the natural pollination behaviour differs between tall and dwarf coconuts. The tall coconuts are considered as cross pollinating with their temporal separation of the male and female phases while the dwarf coconuts are naturally self-pollinating due to the overlapping of male and female phases of the same inflorescence [1]. However, it is speculated that there may be a certain percentage of self-pollination in tall palms as a result of inter-spadix self-pollination especially during the periods of high inflorescence emittance. This phenomenon can have negative effects on open pollinated seedling production because, the resulting progeny can suffer from inbreeding depression. Accordingly, this study was conducted to determine the probability of inter-spadix self pollination by the overlapping of female phase with the male phase of the successive inflorescences.

Materials and Methods

Experimental site

This experiment was carried out at the Isolated Seed Garden (ISG), *Ambakelle* (coordinates: 7° 41'27" N, 79° 53' 48" E) belonging to the Coconut Research Institute of Sri Lanka. The seed garden is situated in *Puttalam* district and belongs to the agro-ecological region, Intermediate low country (IL3).

Data collection

A total of 80 Sri Lankan tall coconut palms were selected to collect secondary data on inflorescence emergence and the initiation and completion of male and female phases of each inflorescence of each palm over four consecutive years from 2016 to 2019. The following information were extracted from the available data.

- (i) The number of inflorescences produced in each month on each palm
- (ii) The number of palms produced two or more inflorescences in each month
- (iii) The number of palms with potential to overlapping of the female phase with the male phase of the successive inflorescence
- (iv) The number of days which the female phase and the male phase of successive inflorescences overlap.

The extracted information was interpreted using basic statistics, means and the monthly trends in inflorescence production and overlapping of female phase with the successive inflorescence were analyzed using the analysis of variance using the statistical software package Minitab version 14.0.

Results and Discussion

Using the gathered information, the annual inflorescence emergence of each palm was calculated. In contrast to the general expectation of one inflorescence per month, the average annual inflorescence production of selected population was recorded to be 16 inflorescences per palm. During the 3-year period, the highest annual inflorescences production recorded in the selected population was 22 inflorescences per palm while the lowest annual inflorescence production was 11 inflorescences per palm. The percentage of the palms opening more than one inflorescence in each month was calculated to identify the monthly trend in inflorescence production (Figure1).

Notable peaks were observed during July/August period whereas a lower percentage of palms opened multiple inflorescences in October and December periods. The percentage of palms that produced more than one inflorescence was as high as 80% in August 2017.

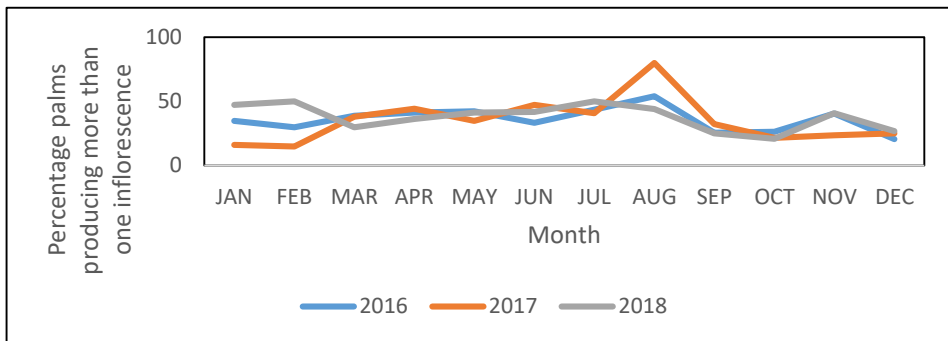


Figure 1. Percentage of palms opening more than one inflorescence during 2016-2018

Analysis of Variance procedure revealed a significant difference in the inflorescence opening rates with a higher percentage of palms producing more than one inflorescence during the period from April to August. A significantly low percentage of palms ($\alpha=0.05$) were emitting multiple inflorescences, in the month of October (23%) and December (24%). A high percentage of palms (59%) produced more than one inflorescence in the month of August (Figure 2).

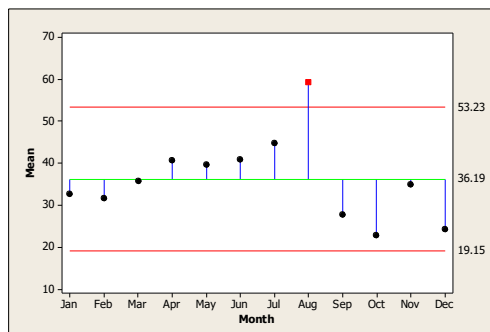


Figure 2. Means of percentage palms producing more than one inflorescence per month during 2016-2018

Generally, a higher rate of inflorescence emittance can be observed in high yielding palms during favourable climatic periods of the year [1]. Usually, these vigorous tall palms produce inflorescences frequently during the peak production months (April–June) and this observation is confirmed with the above results with a shift of the favorable climatic period extending towards August. The higher frequency of emittance of inflorescences in a single palm increases the probability of overlapping of the female phase of one inflorescence with the male phase of the successive inflorescence allowing inter-spadix pollination within the same palm.

The same population of palms were observed for overlapping of successive inflorescences and a total of 864 overlapping inflorescences (Table 1) were recorded during the said period. Out of these 864, the female phase overlapped with the male phase of the successive inflorescence in 758 instances (86% of the total). The period of overlapping varied from a single day to the total duration of the female phase. As shown in Table 1, in about 50% of the incidences the overlapping duration exceeded 4 days indicating a very high probability of inter-spadix pollination within the same palm or self-pollination in some of the years.

Table 1. Summary of the inflorescences with overlapping female and male phases of successive inflorescence

Year	2016	2017	2018	2019
Incidence of overlap	344	251	172	117
% of incidences with single day overlapping	18	19	16	9
% of incidences with 2 days overlapping	13	6	10	11
% of incidences with 3 days overlapping	10	12	8	14
% of incidences with >4 days overlapping	42	47	53	58

Eventhough, Sri Lankan tall coconut populations are considered to be predominantly cross-pollinating [1], our results revealed a higher probability for selfing, in the selected high yielding palms at ISG, *Ambakelle*. The resulting self-pollination can cause homozygosity of deleterious alleles in the subsequent generation, which may result in a progeny suffering from inbreeding depression.

The pollen output of a healthy palm is approximately 111,000 to 221,000 grains [2] and the viability of a pollen in the atmospheric conditions is about 1-2 days [3]. Since the female flowers keep receptive for 1-4 days [4] there is a very high possibility for inter-spadix self-pollination. Furthermore, it has been found that pollen catches during March and April are significantly higher than the other months of the year [2]. Generally, this period corresponds with the period of increased nut yields and vice versa. Therefore, with this background there is a higher chance of creating homozygosity in the upcoming progeny which can lead to inbreeding depression.

In Sri Lanka, most of the planting material for the National Replanting Programme, is produced from a pool of about 50,000 elite mother palms selected from the estates and experimental stations [5]. As our study revealed, it is important to pay attention towards the selfing rates of these selected mother palm pools. In elite palms a higher selfing rate, usually reduces the yield of fruits

from 20 to 30% in a single generation due to selfing [1]. In tall types, selfing generally induces an average yield decline of 15–30% without improving the homogeneity of the population [1].

Conclusions and Recommendations

This study revealed a higher potential for self- pollination between the successive inflorescences of the tall coconut palms especially during the peak inflorescence emmitance months of April to August. This phenomenon might cause inbreeding depression in the successive progeny that could result in weak palms among the tall coconut populations in Sri Lanka. Therefore, it is important to implement measures to restrict or minimize selfing during the open-pollination process. One of the simple options that could be used is, avoiding the harvesting of seed-nuts during the peak month within the year where the inflorescence emmitance is the highest. The second alternative would be to emasculate the second inflorescence of the two successive inflorescences to minimize the overlapping of pollen dehiscence and stigmatic receptivity.

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BORON STATUS OF ADULT COCONUT PALM UNDER DIFFERENT FERTILIZER SOURCE COMBINATIONS: A CASE STUDY

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Introduction

It is very important to apply adequate amount of fertilizers to the coconut palm to balance nutrients in palm to achieve high yield.

Coconut palm necessitates light, water, and about 20 elements to support all its biochemical requirements. These 20 elements are essential to the palm and classified as macronutrients and micronutrients according to the nutrient requirement. Major nutrients such as carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur are considered as macronutrients which are essential for the growth, maintenance, and yield production. Micronutrients are the essential elements that are required in small quantities but directly affect for the performance of the coconut palm.

Micronutrients which include Boron, Chlorine, Copper, Iron, Manganese, Molybdenum, Nickel and Zinc. Out of all micronutrients, boron (B) is unique for the coconut palm as it is known to be one of the most yield limiting factors [1]. Boron is an element important for cell Division and elongation, nucleic acid metabolism, respiration, carbohydrate metabolism, phenolic compounds and lignin biosynthesis, nitrogen metabolism, growth hormone and enzyme metabolism and more importantly for reproductive physiology. Removal of B from adult coconut palms through all plant parts are reported to be 0.15 kg ha⁻¹ yr⁻¹ [2]. Therefore, different rates were introduced in different countries. In Brazil, boron is applied to young coconut palms at the rate of 30 g of borax to 4th leaf axilla. In Kerala, 160 g of borax per palm is recommended as the optimal dose for soil application in coconut growing soils [3]. According to Tamil Nadu Agricultural University, for 1-year old coconut seedlings 5-10 g of borax /plant per year, for 2-3 years old seedlings, 15-20 g of borax /plant per year and for the palms which are 4 years old and more than 30-50 g of borax /plant per 2 years are recommended. It is also known that B incorporation into soil is more effective than foliar techniques [1].

On one hand, major nutrition status of coconut palm is determined from the nutrient analysis of 14th frond which is physiological mature frond of the coconut

palm. On the other hand, it was documented several other fronds, such as 9th, 6th for the micronutrients.

However, effects of borax on soil application to the adult coconut palms under Sri Lankan conditions and the most representative frond for the boron status in adult coconut palms are yet to be confirmed. Therefore, this experiment was initiated to evaluate the nutrient status before and after application of different fertilizer combinations with Borax and to find out the best frond to represent the boron status in adult coconut palms.

Materials and Methods

This experiment was conducted at Poththukulama Research station of Coconut Research Institute of Sri Lanka Puttalam which belongs to the IL3 agro ecological region. The experiment was laid in Randomized Complete Block Design (RCBD) with four replicates having 4 palms per plot. The treatments were applied according to the Table 1 which was commenced in 2021 January.

Table 1. Treatment combination

Treatment	Application Rate / palm / year			
T1	Full basal dose only			
T2	Full basal dose + Borax (50 g)			
T3	Half basal dose + Cattle manure (15 kg)			
T4	Half basal dose + Cattle manure (15 kg) + Borax (50 g)			

Application	Application Rate / palm /year			
	Urea (g)	TSP (g)	MOP (g)	Dolomite (g)
Full Basal Dose	800	400	1600	1000
Half Basal Dose	400	200	1400	500

Leaf samples were collected from 6th and 14th fronds just before treatment application and 6 months after treatment application. Three leaflets on either side were collected from the middle of the frond and ekels were removed. Middle portion of the leaflets were taken for sampling. Collected samples were first cleaned with tap water and distilled water. Cleaned samples were oven dried at 70 °C for 3 days and, samples were ground. Then powdered plant material (1.00 g) was placed in muffle furnace (CARBOLiTe-cwF 1100) at 550 °C for two hours and

dissolved in 0.63 mol L⁻¹ H₂SO₄. After that aliquant (2. 00 mL) of extract was measured and added 6.00 ml of mix reagent of buffer masking agent and Azomethine-H. The absorbance was recorded at 420 nm using spectrophotometer (Thermo EvoLuTion201) and finally, concentrations were calculated. Data were analysed using General Linear Model with Minitab 17 statistical software at 5% significant level and at 95% confidence level. Before treatment application, significant differences were shown in the B levels of the effective palms selected for different treatments. Therefore, B levels before the application of treatments were taken as covariates.

Results and Discussion

Even though B levels were measured in the leaf samples taken from both 6th and 14th fronds, B levels in 14th frond found to be distributed widely with the range of ± 6.07 standard deviation and were not appropriate to be taken as the representative frond to interpret the B status in adult palms. Therefore, levels in 6th frond were considered as the most representative frond for B status in adult palms. Boron content of 6th frond before and after treatment application is presented in Table 2.

Table 2. Boron status of 6th frond before and after treatment application

Treatment	Fertilizer Applied / palm / year			B (ppm)	
	Basel dose	Cattle Manure (Kg)	Borax (g)	Before Treatment Application	After Treatment Application
T1 (Control)	Full dose	0	0	7.71 ^b	8.35 ^d
T2	Full dose	0	50	8.89 ^{ab}	11.76 ^c
T3	Half dose	15	0	8.71 ^{ab}	12.66 ^b
T4	Half dose	15	50	9.30 ^a	13.81 ^a
p Value				0.005	0.000
CV				13.86	19.15
SD				1.20	2.24

Means that do not share a letter are significantly different

According to the table 2, before application of treatments, B levels of 6th frond in all the effective palms were below the critical level. Mathew et al., in 2018 stated that, the critical boron level in leaves of coconut palms grown in Kerala, India was

11.50 ppm and 13.27 ppm according to Cate and Nelson graphical (CN) method and the quadratic plateau response (QP) method respectively [3].

Table 2 reveals that Boron levels of 6th frond in the palms treated with T2, T3, and T4 are significantly higher than the control which was without any organic source / Borax. The highest leaf Boron level (13.81 ppm) was noted in the palms treated with half basal dose + cattle manure (15 kg) + Borax (50 g). Hence, this indicates that B uptake by the palm is facilitated in the presence of organic source. In addition, the increased B level in the 6th frond indicates the enhanced availability of B in soil for palm to take up although Borax was not added. The application of Borax, Borax + organic source, or organic source without Borax to soil could enhance B uptake by the coconut palm and further facilitation of B uptake was observed with Borax with organic source.

Conclusion and Recommendations

According to the standard deviation of leaf B status of the 6th (± 2.237) and 14th (± 6.066) fronds, 6th frond is better suited to determine B status of adult coconut palm. Data of the 6th frond reveals that, B level of all the selected palms were below the critical level (11.50 ppm–13.27 ppm) before the application of treatments and after application of treatments, except in control, B level of all the other palms have reached and reached above the critical level of B. Application of cattle manure has also increased the B level in the leaves. In order to supply the sufficient amount of B to the adult coconut palms where the B levels in the leaves are below the critical level, treatment 2, 3 and 4 can be recommended and the treatment 4 will be the most suitable practice. The palm treated with Borax only, Borax + organic source or organic source only has shown increased B levels in the 6th frond significantly over the control of without organic source. Furthermore, in the presence of organic source in soil facilitates B uptake with borax.

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ENHANCEMENT OF STABILITY AND BIOACTIVITY OF BROWN SEAWEED PHLOROTANNINS THROUGH ENCAPSULATION

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Introduction

The brown algae produce a family of polyphenols called phlorotannins which have proven therapeutic properties such as anti-cancer, antioxidant, antidiabetic, anti-allergic activities. Thus, there exists the potential of incorporating phlorotannins in making functional food and nutraceuticals which may help mitigate the risks of prevalent chronic diseases. However, direct incorporation of bioactive agents into food seems limited in the food industry as they undergo deterioration and loss of activity upon food processing, storage, and digestion. These limitations are overcome by the encapsulation process which preserves bioactive compounds. Phlorotannins are metabolized and absorbed, predominantly in the large intestine while chitosan; the non-digestible polymer gets degraded by colonic microbial enzymes. Thus, chitosan would be an acceptable encapsulating material for the colon-targeted delivery of intact phlorotannins.

In this study, phlorotannins are isolated from brown algae, *Sargassum ilicifolium* (Turner) C. Agardh 1820, and encapsulated in a chitosan- tripolyphosphate envelope. The storage and processing stability of encapsulated phlorotannins are assessed relative to the free form of phlorotannins. A carrier; jelly is fortified with encapsulated and non-encapsulated phlorotannins following their assessment in terms of their bioactivity retention upon *in vitro* gastrointestinal digestion and colon fermentation.

Materials and Methods

Extraction of phlorotannins

The protocol for phlorotannin extraction was referred to Gall *et al.* [1] with few modifications. 15 g of seaweeds; oven-dried at 50 °C was solvent-extracted with 30% ethanol. The extract was centrifuged at 6000 rpm for 10–20 min and discarded the pellet. All alcohol and most of the water were evaporated down to about 40 mL using a rotary evaporator and freeze-dried the remaining aqueous extract.

Semi- purification and identification of phlorotannin fraction

The freeze-dried extract was semi-purified using hexane, dichloromethane, ethanol, acetone, ethyl acetate in order, as described in Gall *et al.* [1].

Phlorotannins in ethyl acetate were then extracted to deionized water and then freeze-dried. ¹H NMR was then performed for the freeze-dried powder to identify the purified phlorotannin fraction.

Quantification of phlorotannins

Folin-Ciocalteu (FC) method was employed as mentioned in Gall *et al.* [1] with few modifications. Absorbance was read at 620 nm. Contents of phlorotannins in each fraction resulted from semi-purification were stated in mg g⁻¹ of extract.

Preparation of phlorotannins encapsulated particles

Encapsulation was carried out following Cerchiara *et al.* [2] with few modifications. 400 mg of freeze-dried phlorotannin powder was dissolved in 60 mL of 0.20% (w/v) chitosan solution prepared in 0.5% (v/v) acetic acid. The suspension resulted from the addition of 0.15% TPP solution was centrifuged at 6000 xg. Pelleted microparticles upon centrifugation were freeze-dried.

Fourier Transform Infrared (FTIR) Spectroscopy analysis

The interactions between chitosan-TPP as well as core-wall material in microencapsulated were investigated by analyzing pure phlorotannin, chitosan, TPP, and formulated particles through FTIR Spectrometer.

Determination of storage stability

Storage stability of encapsulated and non-encapsulated phlorotannins was investigated based on loss of total phlorotannin content (TPC) with time under three commonly used storage environments; -18 °C freezing, 4 °C refrigeration, and storage at 30 °C ± 2 ambient temperature through the FC method as described by Gall *et al.* [1].

Determination of high-temperature processing stability

The two samples of non-encapsulated and encapsulated forms of phlorotannins were placed in a pre-heated oven at 175 °C for 22 minutes to mimic general baking conditions. Compound stability was expressed considering the fraction of TPC that remained after baking.

In vitro gastrointestinal digestion and colon fermentation

The simulated gastric digestion, intestinal digestion, dialysis, and colon fermentation of two jelly samples; fortified with non-encapsulated and encapsulated phlorotannins, respectively, was done as described in Kumari and Gunathilake [3] with few modifications.

Bioassays

The variations in the phlorotannins' bioactivity at each phase of digestion were evaluated through TPC assessment using the FC method as described in Gall *et al.*

[1]. DPPH radical scavenging ability, nitric oxide scavenging assay, and ferrous ion reducing power assay were conducted as described in Kavitha and Palani [4].

Statistical analysis

All determinations were given as mean \pm standard deviations (SD). Statistical analysis was carried out using SPSS 16.0 for Windows software. Significant differences were determined using paired sample t-test. $P < 0.05$ was considered significantly different in all experiments.

Results and Discussion

Identification of phlorotannins

According to Figure 1, the sharp peak observed between 4.5 and 5.0 ppm in the ¹H NMR spectrum of Gall *et al.* [1] corresponds with the D₂O peak at 4.72 ppm of the present study. The peak at 6.7 ppm may have resulted from phenolic protons vibration. The peaks in the 6.0 to 8.0 ppm range may demonstrate the protons of aromatic hydrocarbon groups of phlorotannins in this work.

Quantification of phlorotannins

The crude seaweed extract has only 355.31 \pm 3.72 mg PGE g⁻¹ of phlorotannins, whereas following the semi-purification steps the highest phlorotannin content is observed in the ethyl acetate fraction; 854.38 \pm 48.42 mg PGE g⁻¹. Thus, phlorotannins from the crude extract have concentrated to the ethyl acetate fraction through semi-purification. In compliance, several previous studies also have noted the highest total phlorotannin content in the ethyl acetate fraction compared to each solvent used for fractionating the extract.

Structure characterization by FTIR

The FTIR spectrum of microparticles (Figure 2) is similar to that of pure chitosan, with some exceptions. A new peak has originated at 1624.16 cm⁻¹ while O-H stretching band has more broadened and intensified in microparticles than chitosan due to the formation of new O-H interactions between phosphate groups of TPP with the NH₂ groups of chitosan upon encapsulation. The band at 1556.41 cm⁻¹ has sharpened and intensified due to the formation of more N-H bonds during the encapsulation process. The sharp peak at 1711.72 cm⁻¹ in isolated phlorotannins has disappeared in the FTIR spectrum of microparticles. The intensity of the peaks of phlorotannins at 2924.03 cm⁻¹ and 2853.90 cm⁻¹ also has reduced upon encapsulation. Thus, it is evident that the phlorotannins are existing within the formulated microparticles.

Storage stability

About 30% reduction in phlorotannin content was observed in both encapsulated and non encapsulated forms under four weeks storage at ambient conditions

whereas it was only a 10% reduction in both refrigerated and freezing conditions irrespective to the encapsulation. Similar previous studies also have stated that phlorotannin retention is satisfactory in 4 °C refrigerated conditions compared to 28 °C; corresponding to the phlorotannin retention behavior in this study.

Stability at the high processing temperature

Bakery products and cereals; world's mostly consumed food type are potential carriers of functional ingredients. However, ensuring bioactive stability at baking temperature is vital before fortification. Results revealed 56.4% retention of phlorotannins in encapsulated form and only 10.6% retention in free form proving their inapplicability in free form, but potential applicability in a coated form in functional bakery formulations.

Table1. *In vitro* antioxidant activities of non-encapsulated and encapsulated phlorotannins

Assay	Sample	Undigested	Stage of digestion			
			gastric digestion	intestinal digestion	olysis	fermentation
Total phlorotannin content (mg mL ⁻¹)	Micro	0.660 ±	0.397 ±	0.122 ±	0.840 ±	0.231 ±
	encapsulated phlorotannin	0.460 ^b	0.128 ^a	0.033 ^b	0.327 ^a	0.029 ^a
	Non-encapsulated phlorotannin	3.064 ±	0.410 ±	0.167 ±	0.171 ±	0.173 ±
	encapsulated phlorotannin	0.269 ^a	0.033 ^a	0.020 ^a	0.007 ^b	0.011 ^b
DPPH IC ₅₀ (mg mL ⁻¹)	Micro	0.097 ±	0.144 ±	0.127 ±	0.985 ±	0.033 ±
	encapsulated phlorotannin	0.019 ^b	0.006 ^b	0.010 ^a	0.132 ^a	0.004 ^b
	Non-encapsulated phlorotannin	0.435 ±	0.316 ±	0.082 ±	0.018 ±	0.087 ±
	encapsulated phlorotannin	0.038 ^a	0.075 ^a	0.012 ^b	0.009 ^b	0.010 ^a
NO scavenging (%)	Micro	29.074 ±	49.669 ±	54.814 ±	51.915 ±	71.107 ±
	encapsulated phlorotannin	2.651 ^b	0.296 ^b	0.359 ^b	1.250 ^a	0.165 ^a
	Non-encapsulated phlorotannin	38.018 ±	56.876 ±	69.238 ±	52.902 ±	70.393 ±
	encapsulated phlorotannin	1.813 ^a	0.000 ^a	2.390 ^a	1.219 ^a	0.218 ^a
Ferric reducing antioxidant power (PGE mg mL ⁻¹)	Micro	0.027 ±	0.003 ±	0.011 ±	0.002 ±	0.037 ±
	encapsulated phlorotannin	0.001 ^a	0.002 ^b	0.002 ^b	0.001 ^b	0.000 ^a
	Non-encapsulated phlorotannin	0.014 ±	0.016 ±	0.017 ±	0.038 ±	0.033 ±
	encapsulated phlorotannin	0.002 ^b	0.001 ^a	0.000 ^a	0.000 ^a	0.006 ^a

All data are presented mean ± SD. For a particular bioassay, values in columns with the same letter are not significantly different (P>0.05).

In vitro bioactivity

As provided in Table 1, the TPC and DPPH· scavenging ability of encapsulated form are significantly higher (P<0.05) upon colon fermentation over non-encapsulated form. The findings of Bai *et al.* [5], in which phlorotannins in their non-

encapsulated form have shown a $39.68 \pm 2.96\%$ burst release in simulated gastric fluid and to a final cumulative percentage of $94.72 \pm 1.37\%$ in simulated intestinal fluid backs the findings of the present study. The nitric oxide scavenging property is insignificantly high ($P > 0.05$) in the fermented fraction of encapsulated phlorotannins than free phlorotannins. The reducing power is much prominent in encapsulated phlorotannins in the colon environment compared to its other digestas showing the signs of uncontrolled delivery of phlorotannins in free form.

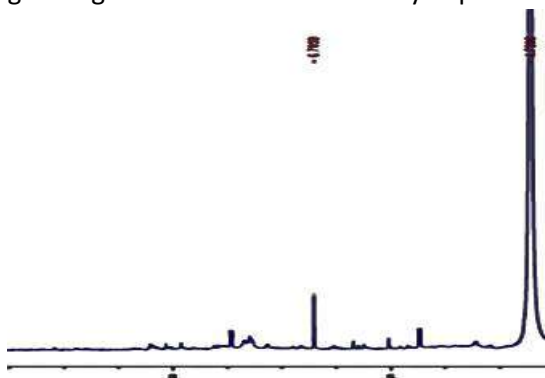


Figure 1. ¹H NMR spectrum of ethyl acetate fraction resulted from polarity-based fractionating in *S. ilicifolium*

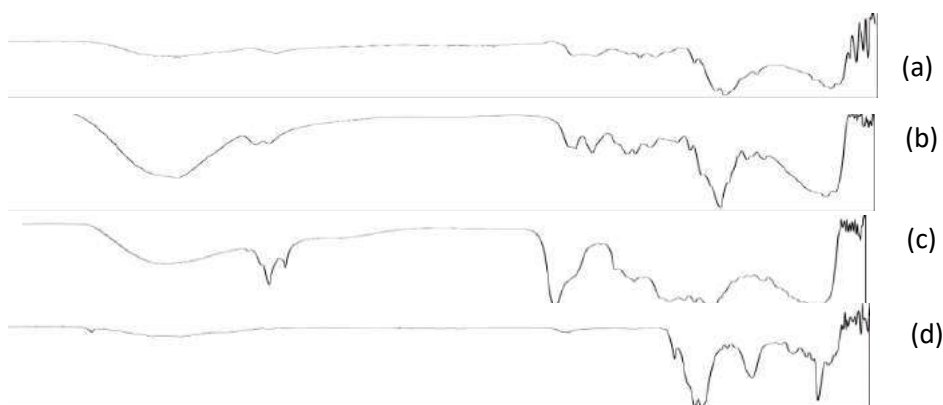


Figure 2. FTIR spectra of phlorotannins (a), phlorotannins encapsulated chitosan-TPP microparticles (b), chitosan (c), and TPP (d).

Conclusions and Recommendations

Solvent extraction and polarity-based fractionation have concentrated semi-purified phlorotannins in the ethyl acetate fraction. FTIR spectra evidence the effective encapsulation of active compounds in chitosan carrier. Low-temperature storage improved the stability of both non-encapsulated and encapsulated forms of phlorotannins while encapsulation significantly improved the phlorotannins' stability at high processing temperatures. The potential for targeted delivery of phlorotannins by encapsulating in the chitosan-TPP carrier was evident from *in vitro* digestion studies. In conclusion, the maximum possible

bioactivity of phlorotannins can be employed by encapsulating chitosan carriers in functional food applications.

Acknowledgement

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EFFECT OF *Spirulina* Sp. IN COMBINATION WITH INORGANIC OR ORGANIC FERTILIZERS ON GROWTH AND YIELD OF OKRA (*Abelmoschus esculentus* L)

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Introduction

Organic agriculture has emerged as a major focus field globally nowadays. The usage of chemical fertilizers alone has several adverse effects on the environment and human wellbeing, in addition, they must be replenished on a regular basis [1]. Organic fertilizers have been shown to improve soil fertility and increase soil biodiversity. *Spirulina* Sp. extract contains nutrients, sugar, and amino acids, as well as growth-promoting bioregulators, vitamins, amino acids, and other secondary metabolites, all of which support plant growth, hence it has also been recommended as a good protein source in animal feeds as well as an excellent alternative to chemical fertilizers [2]. Cattle manure (CM) application to the farmland has been a long-term practice in crop production worldwide. However, the application of cattle manure alone would demand bulk application due to its low nitrogen content. In addition, due to the present pandemic situation, inorganic fertilizer (IN) supply is limited and there is uncertainty of future supply. On this background two experiments were aimed, the first, to find the effect of *Spirulina* Sp. in combination with CM or IN except nitrogen (INEN) on nutrient availability in soil through a laboratory incubation study. The second experiment was to assess their effect on growth and yield of Okra (*Abelmoschus esculentus* L) local variety in a net house pot experiment study.

Materials and Methods

Incubation study

This study was conducted in plastic pots (10 cm diameter, 15 cm height) with 200 g soils per pot. The treatments were T1 (Control), T2 (100% IN), T3 (100% CM), T4 (100% *Spirulina* Sp soil application [SRS] - 6g/kg soil + 100% INEN, T5 (50% IN + 50% SRS), T6 (50% CM + 50% SRS), T7 (100% SRS), T8 (50% IN), T9 (50% CM). Inorganic fertilizer and cattle manure were applied at the Department of Agriculture (DOA) recommendation of fertilizer for Okra. Complete randomized design was used with three replicates for incubation experiment. The treated pots were covered with polythene allowing few holes to ensure aeration and kept under room temperature. The moisture content of pots was maintained at field capacity throughout the experiment. Soil EC, pH were determined using EC meter and pH meter, Total nitrogen was estimated by Kjeldhal method [3]. Phosphorus

content was determined by Vanadomolybdate method [4] and ammonium molybdate- SnCl_2 method was used at the wave length of 660 nm to determine the phosphorous content in soil, potassium content was measured by using flame photometer [5], and total organic carbon content was estimated by loss on ignition method and were measured at two weeks interval until two months of incubation.

Pot experiment

This experiment was conducted in polyhouse located in DL3 agroecological region. Polybag pots having dimension of 20 cm diameter and 45 cm height filled with 10 kg soil. The treatments were T1 (Control), T2 (100% IN), T3 (100% CM), T4 (50% IN + 50% SRS), T5 (50% CM + 50% SRS), T6 (50%IN+ *Spirulina* Foliar spray [SRF]), T7 (50%CM +SRF), T8 (50% IN) and T9 (50% CM). *Spirulina* formulation was prepared using fresh culture grown in the laboratory of Department of Agricultural Chemistry. The foliar spary was applied at two weeks interval. Complete randomized design was used with three replicates for pot experiment. All other management practices were done as per the recommendation of DOA. Growth and yield attributes namely number of leaves, plant height and pod yield were measured at two weeks interval. Data were statistically analysed using ANOVA and mean separation was done using DMRT.

Results and Discussion

The selected physical and chemical properties of soil used in the experiments were pH-6.7, sandy clay loam in texture $\text{EC}-67.4 \mu\text{Scm}^{-1}$, OM - 0.82%, available N-28 ppm, available P-31.8 ppm and available K-61.03ppm. The nutrient content of *Spirulina* sp. was 12% nitrogen, 0.15% phosphorous and 0.55% potassium, while that of cattle manure was nitrogen, 2.1%, phosphorus, 0.51% and potassium 1.21%.

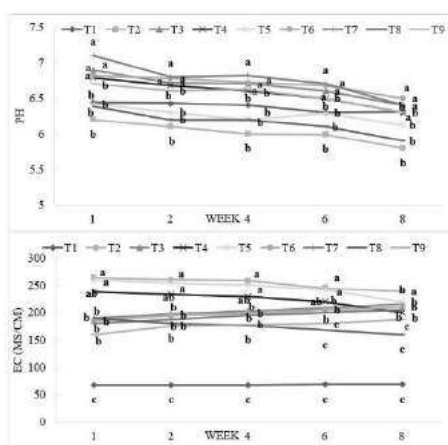


Figure 1. Effect of different treatments on pH and EC with time during incubation

T1 - Control, T2 - 100% IN, T3 - 100% CM, T4 -100% spirulina + 100% INEN, T5 - 50% IN + 50% spirulina, T6 - 50% CM + 50% spirulina, T7 -100% spirulina, T8 - 50% IN, T9 - 50% CM, IN: Inorganic fertilizer based on department recommendation, CM: Cattle manure Same letters are statistically not different by DMRT

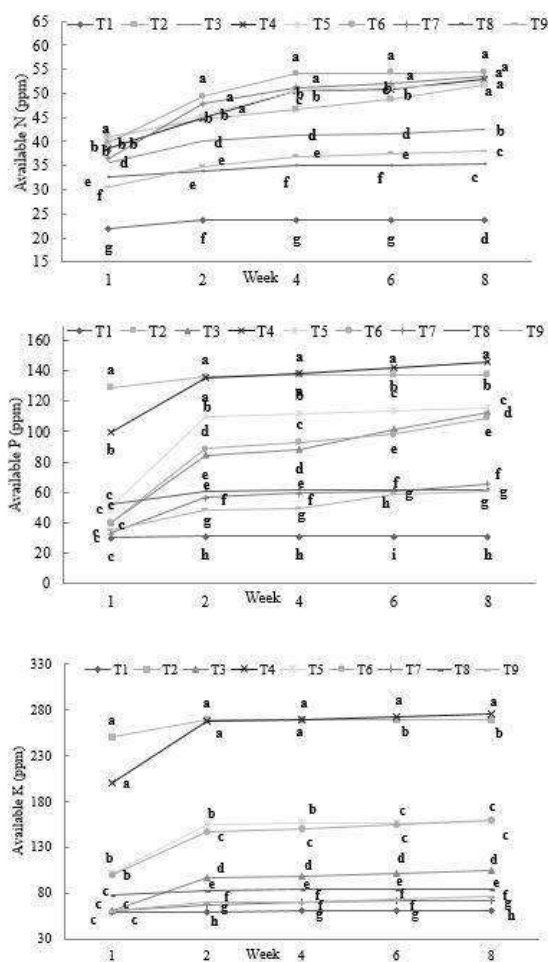


Figure 2. Effect of different treatments on available N, available P, and available K with time during incubation

T1 - Control, T2 - 100% IN, T3 - 100% CM, T4 -100% spirulina + 100% IN (without urea) , T5 - 50% IN + 50% spirulina , T6 - 50% CM + 50% spirulina, T7 -100% spirulina, T8 - 50% IN, T9 - 50% CM, IN: Inorganic fertilizer based on department recommendation, CM: Cattle manure Same letters are statistically not different by DMRT

Results of incubation experiment with different treatments indicated that all treatments shown a pH range (5.8-7.1) which is optimal for plant growth, while T7 (100% SR) shown near neutral pH (Figure 1). Higher EC was shown by T2 (100% IN), however, all treatments recorded desirable EC for plant growth (67.2-264 $\mu\text{S}/\text{cm}$). During first week of incubation T2 (100% IN) showed significantly higher available N, however at 8th week there was no significant difference among T2 (100% IN), T4 (100% SR + 100% INEN), T5 (50% IN + 50% SR), T6 (50% CM + 50%

SR) and T7 (100% SR). This indicates that at 50% rate (3 gkg⁻¹) SPS could complement the nitrogen requirement. In addition, at 100% rate SPS could substitute the nitrogen requirement instead of inorganic fertilizer or cattle manure. Availability of K and P were significantly higher in T4 (100% SR + 100% INEN) and T2 than in other treatments. Moreover, all treatments combined with SRS recorded significantly higher K and P than only 50% inorganic or organic alone treatments. This indicate that SPS could partially substitute P and K nutrition as well.

Table 1. Okra plant height, leaf number at the time of harvest and total yield

Treatments	Plant height (cm)	Total yield (g)	Number of leaves
T1	58.0 ^e	45.0 ^d	10 ^b
T2	78.6 ^d	157.0 ^{bc}	11 ^b
T3	117.3 ^b	206.0 ^{bc}	14 ^b
T4	90.6 ^c	230.0 ^{bc}	12 ^b
T5	101.0 ^b	442.0 ^a	14 ^b
T6	91.3 ^c	238.0 ^{bc}	13 ^b
T7	123.3 ^a	298.0 ^b	20 ^a
T8	75.3 ^d	107.0 ^{cd}	10 ^b
T9	87.0 ^c	118.0 ^{cd}	10 ^b

T1 - Control, T2 - 100% IN, T3 - 100% CM, T4 - 50% IN + 50% SRS, T5 - 50% CM + 50% SRS, T6 - 50% IN + SRF, T7 - 50% CM + SRF, T8 - 50% IN, T9 - 50% CM, IN: Inorganic fertilizer based on department recommendation, CM: Cattle manure, SRS: Spirulina soil application, SRF: Spirulina foliar application. Same letters are statistically not different by DMRT

Table 1 shows the result of plant height and the number of leaves at the time of harvest and total yield of okra in the pot experiment. The highest plant height and number of leaves were observed in T7 (50% cattle manure +Spirulina Foliar Application). In the case of total yield, the highest yield (442 g/pot) was obtained from T5 (50%CM+SRS) and the difference was statistically significant compared to all other treatments. The lowest yield (45 g/pot) was obtained from T1 (Control). Except for T1 (control), all other treatments performed either equal or higher than T2 (100% IN). Spirulina extracts contain a large variety of substances and bioactive compounds that can affect plant growth and production. Spirulina has been found to possess nutrients, sugar, and amino acids, as well as produce growth-promoting bioregulators, vitamins, amino acids, and other secondary metabolites, all of which support plant growth [3]. The treatment T5 (50%CM+50%SRS) recorded significantly higher yield than other treatments. The lowest yield was recorded from control (T1). Treatment T5 (50%CM+50%SRS) shows best responses in all yield and growth parameters of okra as well as economically viable too. Except for T1 (control), the total yield of other treatments were either equal or higher than T2 (100% IN). This indicates that 50% requirement of P and K and 100% requirement of N of okra could be supplimented by SPS. This study has verified that the use of cattle manure and

Spirulina positively influenced on growth, yield parameters and nutrient availability. In addition, SPS gave comparable yield to recommended fertilizer with or without inorganic fertilizer. The use of cattle manure and SPS helps to decrease the inorganic fertilizer usage while improving the yield of Okra.

Conclusions and Recommendations

The results of incubation study reveal that all treatments shown suitable pH and EC ranges required for plant growth. SPS could substitute the inorganic nitrogen partially at a 50% (3 gkg⁻¹) rate and fully at 100% (6 gkg⁻¹) rate, while it has the potential to improve P and K availability in soils as well. In the pot experiments with Okra, Treatment T5 (50%CM+50%SRS) recorded significantly higher yield than other treatments. In addition, either SPS soil application or foliar application in combination with inorganic or organic at 50% rate yielded equal or higher yield than 100% inorganic fertilizer treatment. This study has verified that *Spirulina* sp could help to decrease the inorganic fertilizer usage without affecting the yield of Okra.

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MODELLING AND OPTIMIZATION OF POLYPHENOL EXTRACTION FROM FLOWERS OF *Ocimum sanctum*

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Introduction

Ocimum sanctum belonging to the family Lamiaceae is a shrub widely grown in South Asian countries. Various parts of this plant is well known for its therapeutical potential. Traditionally, edible flowers of this plant has been added to salads as flavoring agents and used in ayurvedic treatments for the management of various disease conditions such as gastric, hepatic, cardiovascular and immunological disorders [1]. Bioactive compounds present in this plant has been identified to contribute to control disease development by exerting various bioactivities such as antioxidant, anti-inflammatory and anti-diabetic activities.

Extraction of bioactive compounds from different plant matrices is a crucial step for these compounds to exert bioactivities effectively. Various techniques including solid-liquid extraction, microwave extraction and supercritical fluid extraction are being employed to extract bioactive compounds from plant matrices. However, the solid-liquid extraction is the most common technique applied and has been considered as a reliable and quiet efficient method [2]. The efficacy of the solvent extraction is influenced by various factors including extraction time, temperature, solvent concentration and the composition of the matrix. Thus optimizing the extraction conditions specifically for each matrix would be more useful to effectively utilize the plants. Response surface methodology (RSM) is a statistical method based on regression analysis that is applied to describe the effect of different independent variables and facilitates optimization of analyzed factors on a particular process. This tool enables users to maximize or minimize the process variables as it evaluates multiple responses simultaneously. The present study aims to apply the RSM approach to simultaneously optimize the process parameters in order to maximize the yield of total phenolics and total anthocyanin content along with maximum antioxidant activity from *O. sanctum* flowers.

Materials and Methods

Fresh *O. sanctum* flowers were collected, cleaned and freeze dried. The freeze dried samples were ground and stored at -18 °C until further analysis. The powdered sample (1 g) was mixed with different volumes of ethanol in the range of 20-40 mL, at different concentrations in the range of 40% to 100%. The homogenized samples were exposed to different temperatures (30-60 °C) for varying time period (30-60 minutes). Values were selected based on literature. The obtained extracts were filtered through Whatman filter paper # 1 and stored at -4 °C in the darkness until further analysis.

RSM was selected for the optimization of process parameters. A two-level four factor central composite design, consisting of 31 experimental runs was used to study the effect of four independent variables (X_A = Liquid to solid ratio, X_B = Ethanol concentration, X_C =Temperature, X_D =Time) on the response variables such as Total phenolic content (TPC), Total anthocyanin content (TAC) and DPPH radical scavenging activity. Experimental design was generated using the software Minitab 17. Response surface analysis and Analysis of variance (ANOVA) were used to determine the regression coefficients and the statistical significance of the model terms. The observed values are then fitted into a second order polynomial model as shown in the Equation (1).

Equation 1 ; $Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_{ij}$

Y indicates the response variable, X_i and X_j are independent variables, β_0 is the constant coefficient of the model, β_i , β_{ii} and β_{ij} are regression coefficients of the single effects, quadratic effects and interactive effects of independent variables respectively. Optimization was performed by maximizing the response values. The optimized conditions were validated based on the experimental values. In order to determine this, experiments were performed under the optimal conditions.

TPC of the extracts was determined using the Folin-Ciocalteu assay and the concentration of total phenols was expressed as mg gallic acid equivalents (GAE) per g dry weight (DW) of flowers. TAC was determined based on the pH-differential method and the results were expressed as milligrams of cyanidin 3-glucoside equivalents (cy-3-glu) per gram of DW of flowers. Antioxidant activity of the extracts was measured based on the ability of the extracts to scavenge DPPH radicals and the percentage of DPPH radical scavenging was calculated.

Results and Discussion

Central composite design was used to determine the effect of four process parameters on TPC, TAC and DPPH radical scavenging activity of *O. sanctum* flowers. Modelling of the extraction parameters were carried out using second

order polynomial equation. Overall ANOVA results revealed that all model responses were significant ($p < 0.05$) while the lack of fit was insignificant ($p > 0.05$). The non-significant lack of fit indicates that the model term adequately explains the relationship between the process parameters and model responses.

Effect of extraction variables on TPC

Based on the obtained responses, after the elimination of all insignificant effects of extraction variables, the final model generated for TPC by fitting the second order polynomial, was as follows,

$$\text{TPC (mg GAE / g DW)} = 32 + 1.88X_B - 3.77 X_C - 0.00701 X_B^2 - 0.0204 X_D^2 - 0.039 X_{AB}$$

It was observed that the model p value was 0.020 and lack of fit was insignificant with the p value of 0.68, indicating that the proposed model is well fitted. The linear effect of ethanol concentration and temperature had a significant effect ($p < 0.05$) on the yield of TPC. It was noted that within the temperature range of 45 °C to 70 °C the yield of TPC has increased. This is primarily due to the fact that increasing temperature increases the diffusion of molecules from the flower matrix and also higher temperatures weaken the intercellular interactions and soften the plant tissues facilitating efficient extraction [3]. However, since phenolics are thermolabile compounds, after reaching a particular temperature, degradation and transformation of phenolics leads to a lower TPC. As noted in the quadratic model, it could be seen that ethanol concentration and extraction time had a negative impact on TPC.

Effect of extraction variables on TAC

The proposed model for TAC shown below, had a p value of 0.008 and the lack of fit was insignificant ($p = 0.987$) indicating that the model shown below is well-fitted and reliable to explore further. Considering the linear effects, solid to liquid ratio had a significant ($p < 0.05$) positive effect on TAC. Based on the quadratic model, ethanol concentration and extraction time had a significant ($p < 0.05$) negative effect on TAC. It was noted that when increasing the extraction time above 45 minutes the yield of TAC has decreased. Generally, appropriate extraction time is required for the equilibrium to be reached between the solution in the flower matrix and extraction solvent. The equilibrium concentration is important for the efficient diffusion of phenolics from the flower matrix and the time required to reach the equilibrium concentration varies depending on the phenolic composition of the matrix [4]. However prolonged extraction time can lead to degradation of phenolics leading to lower TPC.

$$\text{TAC (mg cy-3-glucoside / g DW)} = 17 + 0.505 X_A + 0.000682 X_B^2 - 0.00237 X_D^2 + 0.00126 X_{BD} - 0.00405 X_{AD}$$

Also interactive effect between ethanol concentration and extraction time and the interactive effect between solid to liquid ratio and extraction time (X_{CD}) had a significant ($p<0.05$) effect on the yield of TAC.

Effect of extraction variables on DPPH radical scavenging activity

As per the results obtained from ANOVA, the model for DPPH scavenging activity was significant and the model could be used to predict the responses. The generated model with insignificant lack of fit ($p=0.341$) is represented as follows,

$$\text{DPPH scavenging activity (\%)} = 20.2 + 0.026X_B + 0.648 X_C + 0.01074 X_D^2 - 0.00585 X_{AB} - 0.0046 X_{AD}$$

DPPH radical scavenging activity varied from 14.93% to 58.89% and was mainly affected by the ethanol concentration and temperature in the linear model, and by extraction time in the quadratic model. For interactive effect solid to liquid ratio and ethanol concentration and solid to liquid ratio and time showed a significant negative effect on the DPPH radical scavenging activity ($p<0.05$).

Optimization of process variables and model validation

The determination of optimum conditions for the simultaneous extraction of phenolics and anthocyanins and optimum DPPH radical scavenging activity was carried out using Minitab version 17. The optimal conditions were obtained using the desirability function in the scale of 0-1. The desirability value of 1 represents the ideal case and 0 indicates that one or more responses fall outside the desirable range. The generated optimum conditions for maximum TPC and TAC with maximum radical scavenging activity was 55% ethanol, 1:35 solid to liquid ratio at extraction temperature of 45 °C and time 32.6 minutes. An experimental run was conducted with the recommended optimum conditions and the obtained responses for TPC, TAC and radical scavenging activity were compared with the predicted values to study the appropriateness of the response models. The obtained values are presented in Table 1. There was no statistically significant ($p>0.05$) difference between the predicted and experimental values at 95% confidence interval. The outcomes indicate the reliability of the parameters for the extraction of phenolics and anthocyanins with maximum antioxidant activity from *O. sanctum* flowers.

Table 1. Predicted and experimental values of responses under optimum conditions for simultaneous optimization of responses

Responses	Predicted values	Experimental values
TPC (mg GAE/ g DW)	127.35	124.21±2.58
TAC (mg cy-3-glucoside)	2.66	2.53±0.05
DPPH (% scavenging)	76.36	75.23±1.23

Experimental values are expressed as mean±standard deviation

Conclusions and Recommendations

The current study investigated the optimized conditions to enhance the recovery of phenolics and anthocyanins from *O. sanctum* flowers along with optimum radical scavenging activity. The RSM based second order polynomial models were adequate to optimize the ethanol based extraction of antioxidant compounds due to satisfactory ANOVA and descriptive statistics parameters. The findings of the current study may be useful for the food industry and pharmaceutical analysis to directly apply the optimized conditions so that it can reduce the cost and labor intensive process with efficient extraction of antioxidant compounds.

Acknowledgement

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KNOWLEDGE, ATTITUDE AND BEHAVIOR TOWARDS HERBS AS A FUNCTIONAL FOOD: AN ONLINE SURVEY CONDUCTED IN SRI LANKA

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Introduction

Herbs are considered functional foods due to their contribution towards physiological benefits such as prevention of non-communicable diseases (NCDs) beyond the provision of basic nutrients [1]. Bioactive compounds present in herbs are carotenoids, phenolic acids, flavonoids, coumarins, alkaloids, polyacetylenes, saponins and terpenoids among others. These non-nutritive compounds provide a wide range of health benefits such as antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic, anti-carcinogenic and anti-aging effects. They are specifically used to reduce the risk of non-communicable diseases such as type 2 diabetes, hypertension, and dyslipidemia [2]. There is a growing interest in nutraceuticals and functional foods in the global market due to new research on their application, increasing health issues and consumer demand. The demand varies with consumers' interest and background. In Sri Lanka, herbs are used in traditional systems of medicine to treat ailments such as worm infestations, dysuria, polyuria, renal calculi, neuralgia, wounds, jaundice, impotency, diarrhea, dermatitis, muscle sprains, and liver disorders and edible parts of functional herbs are used as food [3]. There are limited published reports in Sri Lanka on determining the levels of knowledge, attitude and behavior (KAB) on functional herbs among the general public. This study was designed to identify the demand for herbs by Sri Lankans. The main objectives of the study were to, determine the levels of respondents' knowledge on herbs; to identify the major purposes of using herbs; and to determine the socio-demographic factors that affect the KAB levels on herbs.

Materials and Methods

Research instrument

The cross-sectional KAB survey was conducted through an online questionnaire prepared on a Google Form. Possible respondents were adults over 20years of age, currently living in Sri Lanka. Required minimum sample size was calculated using a sample size calculator (<https://www.calculator.net/sample-size-calculator.html>) and was 385 at a confidence interval of 95%, based on the total population size of adults in Sri Lanka (Central Bank of Sri Lanka, 2019) Ethical

clearance was obtained from the Ethics Review Committee of Faculty of livestock Fisheries and Nutrition, Wayamba University of Sri Lanka. The questionnaire was pre-tested using a convenient sample of experts in the field of food science and nutrition; academics, dietitians and nutritionists.

Data collection

The pre-tested, self-administered questionnaire was forwarded to possible respondents through open invitations on social media; Facebook, WhatsApp and emails. Questions were on socio-demographics, knowledge, attitudes and behavior on functional herbs. After data checking and cleaning, data from 417 respondents were proceeded for analysis.

Data analysis

Statistical analysis was done using SPSS 26 (Statistical Package for the Social Sciences) and IBM AMOS 23 (Analysis of a moment structures) software. Exploratory factor analysis (EFA) was done using SPSS. The data set was checked for outliers and 12 outliers were removed [4]. EFA with Varimax rotation and maximum likelihood extraction method was run and 5 factors with eigenvalues exceeding 1.0 were obtained. The five factors or latent variables were measured using items, where each question answered by the respondents were considered as an item. Confirmatory factor analysis (CFA) was performed using AMOS. Various fit indices were used to test the adequacy of the model. They were as follows; CMIN/DF 2.457, NFI 0.813, CFI 0.876, RMSEA 0.06. The five factors were named as; knowledge, positive attitudes, negative attitudes, usage to overcome NCDs, usage for wellbeing. The associations between the factors and the socio-demographic profile were tested using AMOS path diagrams. Further, descriptive statistics on MS Excel were used to depict the responses.

Results and Discussion

Socio-demographic profile of respondents

Respondents were representing 22 districts in Sri Lanka, out of which a maximum of 33.3% were from Gampaha district. 55.4% of the total respondents were female and 44.6% were male. More than 90% of the respondents were Sinhalese, while the rest were Tamils, Muslims and others. The majority of 53.2% of the respondents were reading for a bachelor degree as their highest educational qualification, while the others' highest education levels were varying as GCE Ordinary level, GCE Advanced level, Diplomas, Masters degrees or Doctoral degrees. Monthly income of 45.3% of respondents were within the range of LKR 50,000-100,000. The Majority (88%) of the respondents were not in use of medications for NCDs.

Socio-demographic factors affecting the KAB on herbs

KAB on herbs showed significant correlations with the socio-demographic factors; gender, ethnicity, education level and presence or absence of NCDs. According to output by AMOS, the five factors showed significant correlations at percentages as shown in Table 1.

Table 1. Significant correlations and between KAB on herbs as represented by the eight factors, with the socio-demographic profile

Factor	Gender	Ethnicity	Education	NCD presence
1 Knowledge	16%		12%	
2 Positive attitudes	12%			
3 Negative attitudes				
4 Herb usage to overcome NCDs				12%
5 Herb usage for wellbeing	62%	16%	71%	

Studies conducted in various parts of the globe had also revealed that the KAB levels on herbs depend on a variety of factors including gender, race, age, marital status, and employment status, education, level of training, and working experience etc. which support the findings of this research [4,5].

Purposes of herb usage

The study results in Table 2, showed that people used herbs for purposes such as to overcome cold/fever, strengthen the immune system, weight loss etc. which were agreed by previous studies [5].

Table 2. Purposes of herb usage and the percentage of respondents using herbs for the particular purpose

Purpose of using herbs	Percentage of respondents %
During common cold/fever	82.2
For relaxing mind	23.7
To control blood sugar	14.5
To support heart health	15.4
For cholesterol lowering	19.0
To decrease body weight/slimming	25.7
For promoting energy and wellness	32.5
For strengthening the immune system	52.4
To overcome menstrual complications	23.3
To maintain healthy skin, brightening the skin tone	26.2
To maintain healthy hair	28.2

Knowledge on functional properties of herbs

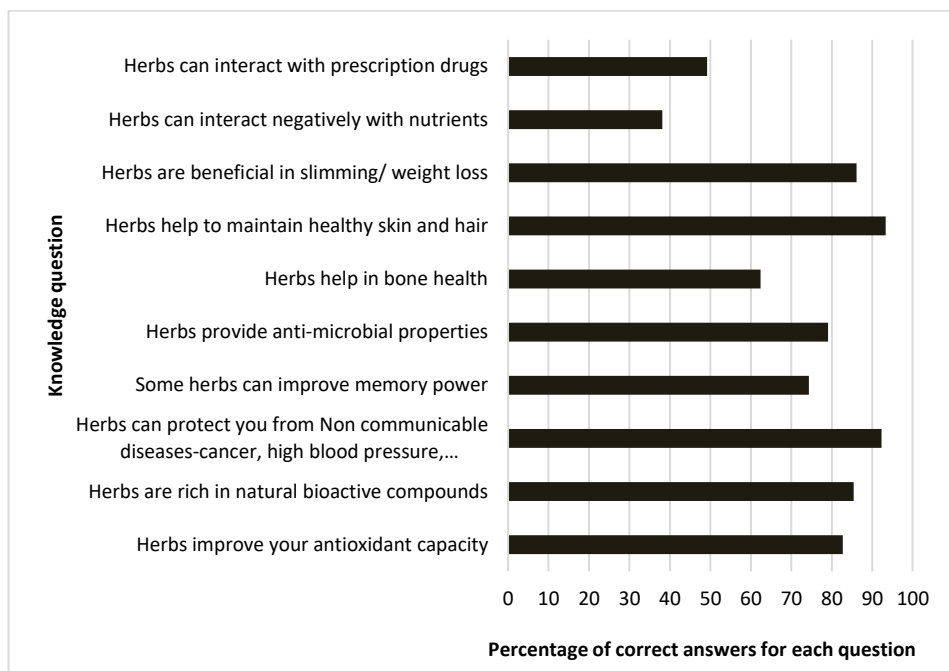


Figure 1. Questions on knowledge of herbs as a functional food, as appeared in the questionnaire and the percentage of respondents giving correct answer for each question

According to the results in Figure 1, only 49% of the respondents knew that herbs can interact with prescription drugs and only 38% knew that herbs can negatively interact with nutrients. This finding agreed with previous studies which stated that respondents had low knowledge on drug interactions, side-effects and toxicities of herbs [5].

Conclusions and Recommendations

Knowledge, attitude and behavior on herbs among Sri Lankan adults significantly correlate to the socio-demographic factors of age, gender, ethnicity, education level and presence or absence of NCDs. Major purposes of using herbs were during common cold/fever and for immune strengthening purposes among others. More than 90% of respondents knew that herbs can protect against Non-communicable diseases. Less than 50% of the respondents had knowledge on herb-nutrient and herb-prescription drug interactions.

This study identified four socio-demographic factors which show significant correlations with KAB on functional properties of herbs. Future research can be conducted to determine the other socio-demographic factors which affect the KAB levels of herbs among Sri Lankans. There are gaps in the knowledge. Approaches can be planned to provide the lacking knowledge and to enhance

herb usage through awareness programs, advertising through media, and providing knowledge from school syllabus, in order to obtain health benefits from the functional components present in herbs.

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PHYSICO CHEMICAL EVALUATION OF SPRAY DRIED MILK POWDER

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Introduction

Milk is a nutrient-dense beverage. Raw milk from healthy cows is meant to have a low bacterial count. Drinking raw milk from healthy cows lowers the risk of disease, but it is perishable. Milk Production (MP) is an excellent option for folks who do not have immediate access to proper refrigeration. MP is obtained by removing the water content of milk. The primary goal of converting milk to MP is to transform a perishable liquid into a product that can be stored for several years without significant quality degradation. MP production has become an increasingly important segment of the dairy industry, and it is expected to continue to grow due to benefits such as better quality preservation, less storage space, and lower transportation costs, which result in appealing economics and convenience during industrial and domestic composite food formulation [1]. Milk powder is typically manufactured by spray drying evaporated milk to improve its shelf life. At room temperature, skim milk powder can be stored for 18–24 months without significant changes in the quality characteristics. Furthermore, milk powders are chosen because they are easier to travel, handle, process, and formulate food goods. Reconstituted or recombined dairy products, as well as bread, confectionery, and meat products, use milk powders. As a result, in the dairy business, better milk powders with high functionality and quality are critical [2]. The study was conducted to determine the physico chemical quality parameters of freshly made spray dried milk powder.

Materials and Methods

Reception of raw milk

This experiment was carried out at the Pelwatta dairy factory-milk Buttala's powder production unit. Bulk cow milk suppliers from Nuwara Eliya, Hatton, Bandarawela, Badulla, Welimada, Dikoya, Sammanturai, Akkaraipattu, Iluppaddichene, Siththandi, and Polonnaruwa districts provided fresh milk.

Following reception, the milk was rechecked for adulterants and microbiological quality using platform testing. Milk was injected into raw milk tanks after the laboratory gave its approval.

Platform and adulteration tests for raw milk

Platform tests such as the alcohol test, and the Gerber fat test were performed on the milk samples. After the platform tests were completed, milk samples were

tested for adulteration using methods such as the Salt Test, Sugar in Milk, Ammonium Sulphate in Milk, Formalin in Milk, Boric Acid in Milk, and Determination of Hydrogen Peroxide in Raw Milk (VODES TEST).

Production process of dried milk powder

A cream separator (SEITAL S.R.L, Italy Model SE 15 X) was used to standardize raw milk, resulting in a milk fat level of about 3.1 percent. After that, standardized milk was boiled for a 15 minutes at 100 °C. In a four-effect falling film evaporator (SSP pvt Limited, India) with a TVR (thermal vapor recompression) system, preheated milk was concentrated under a vacuum. A two-stage drier (SSP pvt Limited, India) with a high-pressure nozzle atomization technology was used to dry concentrated milk. Dried milk powder was chilled to room temperature before being packed into 25 kg plastic-lined multi-wall bags ((thickness 60µm and polyethylene and metallized polyester laminate sachets). The products were then stored at a temperature between 25 and 28 °C.

Physico-chemical properties

Using the air oven method, the moisture content was determined. Titrable acidity was measured by titrated against 0.1 NaOH, bulk density was calculated by measure the mass of milk powder occupies a fixed volume, and solubility index in freshly manufactured milk powder were all measured.

Statistical analysis

Treatment means were compared using the Duncan's Multiple Range Test. To see if there were any significant differences between the data, an ANOVA test was run at a 0.05 level ($p < 0.05$). Minitab 18 was used for statistical analysis. Each variable was replicated three times during the experiment.

Results and Discussion

Table 1. Quality parameters of raw milk used in the experiment

Raw milk Properties						
Centre	Fat (%)	SNF (%)	Titration acidity (%)	Adulteration	H ₂ O ₂	T (°C)
LN-9355	4.60±0.03	8.16±0.12	0.16±0.01	Neg	Neg	10
LH-3517	5.71±0.06	8.57±0.17	0.17±0.02	Neg	Neg	09
LL-3471	4.13±0.02	8.13±0.10	0.16±0.01	Neg	Neg	11
LL-0426	4.20±0.05	8.70±0.18	0.17±0.02	Neg	Neg	09
43-6016	5.22±0.05	8.93±0.19	0.16±0.01	Neg	Neg	10
Ruwansi	4.30±0.01	8.24±0.13	0.16±0.01	Neg	Neg	08
Kappatipola	4.52±0.03	8.20±0.11	0.17±0.02	Neg	Neg	09

SNF- Solid Non Fat, Neg –Negative, T – Temperature

Values are the means of triplicate

All selected bulk samples of raw milk were given negative results to adulterants. These results clearly showed that the volume of raw milk used in the experiment were free from adulteration.

Effect of dryer plant operating parameters on the quality of dried milk powder

Following quality parameters of dried milk powder such as bulk density, insolubility index and moisture content were considered.

Bulk density

Bulk density is a crucial economic, commercial, and functional attribute. The bulk density increased in the beginning and reduced in the conclusion, as shown in Figure 1. With an increase in temperature, the bulk density and degree of shrinkage increased in a trend. Overheating of the milk, expansion of trapped air bubbles, creating cracks in the particle surface, a higher combined vacuole volume, and a low mean density of the powder particles were all caused by higher outlet temperatures (95 °C to 105°C). As a result, such milk powder had a low bulk density. If the total solids concentration in the milk to be dried is low, a high output air temperature may result in high porosity of the powder particles [3].

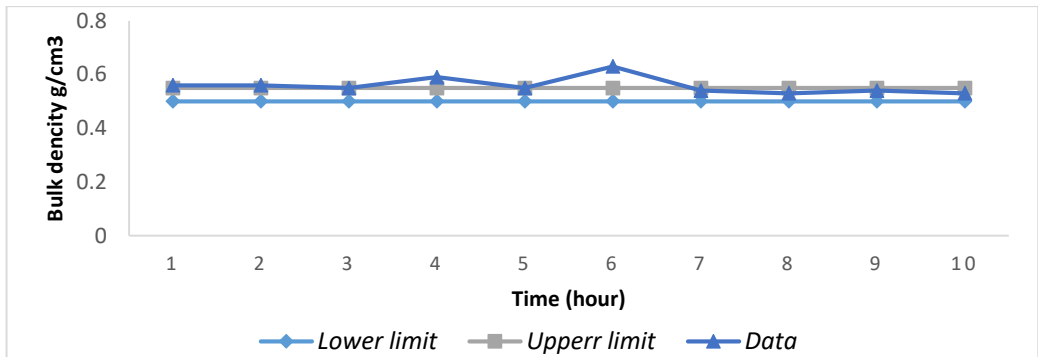


Figure 1. Behavior of bulk density with different samples at different time intervals values are the mean±standard deviation (n = 3)

Bulk density should be in the range of 0.5-0.55 g/cm³ according to plant designs. The data from the experiments came close to the range, although only a handful of them gained 0.63 and 0.59 values. These variances can be regarded as a minor increase. As a result, the bulk density quality parameter was satisfied.

Insolubility index

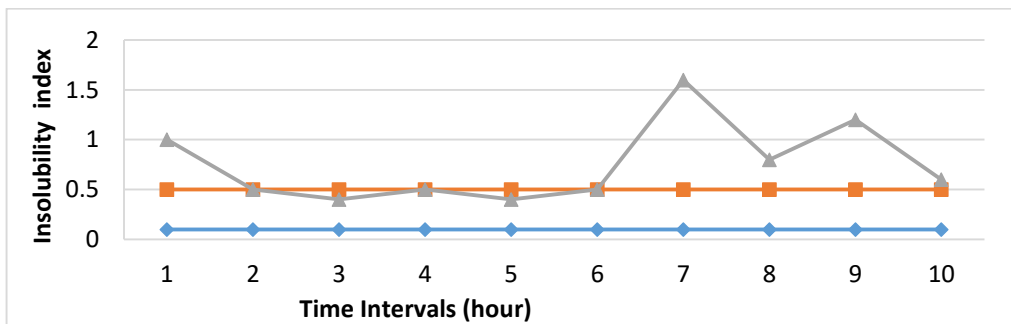


Figure 2. Behavior of insolubility index with different samples at different time intervals values are the mean±standard deviation (n = 3)

Figure 2 clearly demonstrated that the data did not behave in the suggested plant design range in this investigation. In this situation, certain powder samples had a higher Insolubility Index (i.e. poor solubility). The high insolubility score could be due to a variety of factors. Insolubility index is usually caused by denatured caseins or exceedingly complex combinations of casein-whey protein and lactose. Insolubility Index will be high in low-quality milk with a high growth of lactic acid, i.e. bacterial activity, because any lengthy heat-treatment will cause permanent protein denaturation, notably of caseins. High temperatures during the evaporation of the concentrate will induce a significant age-thickening, resulting in increased viscosity and poor atomization, i.e. high temperatures during the drying [4].

Moisture content

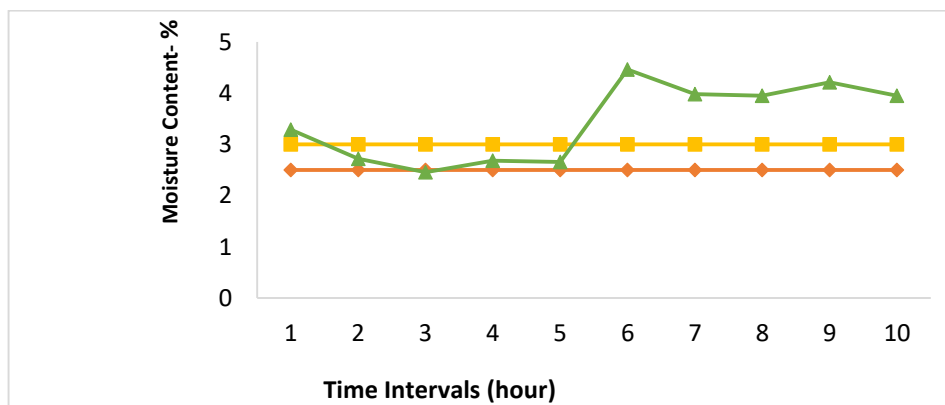


Figure 3. Behavior of percentage of moisture content with different samples at different time intervals
values are the mean±standard deviation (n = 3)

The moisture content will affect the powder's ability to hold its shape. The allowable suggested moisture content limit, according to the standard plant design parameter, is 2.5-3%. However, as shown in Figure 3, moisture content fluctuations were observed in all samples, indicating a serious problem with milk powder quality. As a result of the high moisture content, the proteins will desaturate and the lactose, which is found in an amorphous state, will crystallize, causing the free fat in whole milk powders to increase, resulting in fat oxidation. The output is utilized to adjust the outlet temperature by regulating either the feed pump or the heat provided to the Vibro-heating fluidizer's section, and the reflection from the sample is directly proportional to the moisture content [4].

Conclusions and Recommendations

The quality of raw milk did not seem to vary much based on the results of the experiments. Bulk density of freshly made milk powder was nearly within the range of plant design. Insolubility index of some powder samples exceeded the recommended plant designs range. According to the plant's approved design, the ultimate outflow powder moisture should be 3.0-3.5%. The experimental results revealed that moisture was supplied rather than removed during the cooling section. As a result of this inappropriate moisture removal, the solubility of milk powder was badly harmed while lowering the agglomeration of powder particles. The fluidized bed dryer's temperature gradient and powder temperature change could be the cause. Milk powder quality and storage will be harmed by high moisture content.

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ANTI-OXIDANT POTENTIAL IN SEEDS OF LOCAL VARIETIES OF *Vigna unguiculata* (COWPEA)

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Introduction

Nutraceuticals are foods, herbal products or processed/genetically modified foods which provide nutrition as well as considerable health impact in consumers. Nutraceuticals play an important role in improvement of health through prevention of diseases. Therefore, recently nutraceuticals have been gained attention globally and it is recommended to promote nutraceuticals among populations to ensure a healthy life [1].

Among different natural resources, plants have been recognized as most reliable nutraceuticals due to their production of a variety of bioactive compounds as secondary metabolites. These compounds are not essential for the survival of the plant but involved in adaptation to the environmental conditions in which the plant grows. Hence, the types and the quantity of secondary metabolites synthesized by a particular plant is determined by the different environmental conditions it undergoes during its life time. Thereby, even the members of same species which grow in different countries or regions may contain different types of bioactive compounds in different quantities due to seasonal variations. Hence, the health impact exerts by plant products from same species growing in different countries may vary [1].

Legumes have been identified as nutraceuticals due to high nutritional value as well as the pharmacological properties they exert. Among them *Vigna unguiculata* (cowpea) is a popular pulse growing in semiarid regions across Africa and Asia which exhibit higher tolerance for sandy soil and low rainfall. Thereby, cowpea is a legume crop which is mainly cultivated in rural areas of Sri Lanka, which is considered as an inexpensive source of proteins [2].

Field Crops Research and Development Institute of Sri Lanka (FCRDI) released several local varieties of cowpea with high yield, which have been cultivated during past years in Sri Lanka. The scientific data available on nutrition composition of these varieties proved that they are relatively rich in nutrition with high protein content. However, the pharmacological properties of these varieties have been overlooked [3].

Under certain circumstances, free radicals are produced in excess within in human body which may leads to sever diseases. Antioxidants can neutralize or scavenge the highly reactive free radicals and reduce the damage induced within human body. Therefore, the routine consumption of antioxidants is recommended to prevent and alleviate the diseases caused due to development of oxidative stress within the body including diabetes, cardiovascular diseases, anti-inflammatory diseases, etc. The sources of antioxidants can be natural or artificial. Fruits and vegetables are mainly recognized as sources of anti-oxidants and therefore, recommended to add in the daily diet in order to maintain human health [4].

The objective of the present study was to reveal the anti-oxidant potency of the seeds of local cowpea varieties with the purpose of promoting them as nutraceuticals.

Materials and Methods

Seed samples of four popular local varieties of cowpea known as Waruni, Dhawala, MICP 1 and Bombay were collected from Field Crops Research and Development Institute of Sri Lanka (FCRDI). Methanol extracts of each cultivar was prepared separately by soaking 5 g of the fine seed powder in 50 mL of methanol water for 7 days. Then the extracts were filtered and the supernatant was stored under 4 °C until it was used.

The antioxidant potency of the seed samples was evaluated by the means of DPPH radical scavenging assay, Ferric reducing antioxidant power assay and the Nitric oxide scavenging assay using standard methods. In addition, the total phenolic and total flavonoid content were estimated [5].

All the assays were performed in triplicates. The results were statistically analyzed by SPSS 20.

Results and Discussion

The results of the DPPH radical scavenging assay, Nitric oxide scavenging assay, Ferric reducing antioxidant power assay, the total phenolic and total flavonoid content are presented in Table 1.

Among the seed samples tested, significantly higher ($p < 0.05$) DPPH radical scavenging activities were exerted by the two varieties called Waruni ($82.38 \pm 0.01\%$) and Dhawala ($82.79 \pm 0.02\%$). The highest ferric reducing antioxidant capacity was exhibited by the seeds of Bombay (0.72 ± 0.01 mg of ascorbic acid/g of the extract). MICP 1 seeds (1.21 ± 0.04 mg of gallic acid/g of extract)) and Waruni seeds (1.20 ± 0.03 mg of gallic acid/g of extract)) demonstrated higher nitric oxide scavenging activity compared to the other seed samples. Among

tested seed samples, Waruni (0.04 ± 0.02 mg of quercetin/g of extract) exhibited the highest total flavonoid content. When considering observed total phenol content, the seed samples of Waruni (1.30 ± 0.01 mg of gallic acid/g of extract) and Bombay (1.41 ± 0.02 mg of gallic acid/g of extract) demonstrated significantly higher ($p < 0.05$) amount.

Table 1. The DPPH radical scavenging capacity, Ferric reducing antioxidant power, Nitric oxide scavenging capacity, the total phenolic content and total flavonoid content of seed samples of four local varieties of *Vigna unguiculata*

Variety	DPPH (%)	FRAP (mg of ascorbic acid/g of the extract.	NO Scavenging Activity (mg of gallic acid/g of extract)	Total Flavonoid Content (mg of quercetin/g of extract.	Total Phenol Content (mg of gallic acid/g of extract)
Waruni	82.38 ± 0.01	0.58 ± 0.04	1.20 ± 0.03	0.04 ± 0.02	1.30 ± 0.01
Dhawala	82.79 ± 0.02	0.32 ± 0.01	0.97 ± 0.04	0.01 ± 0.01	0.88 ± 0.03
MICP 1	51.61 ± 0.01	0.44 ± 0.02	1.21 ± 0.04	0.01 ± 0.04	0.68 ± 0.04
Bombay	50.54 ± 0.03	0.72 ± 0.01	1.10 ± 0.02	0.02 ± 0.02	1.41 ± 0.02

Data represented as mean \pm SE

The results of the present study suggest that the DPPH and NO scavenging activity of the seed sample of Waruni is mainly exert by flavonoid and phenols present in the methanol extract, while the DPPH scavenging activity of the seeds of Dhawala may exert by biocompounds other than flavonoid and phenols. The observations of the study reveal that the NO scavenging activity of the variety called MICP 1 also seems to be exert by other secondary metabolites except flavonoid and phenols. Ferric reducing antioxidant power of variety known as Bombay may depend upon the phenolic compounds present in the methanol extract.

The results revealed that all four seed varieties possess remarkable antioxidant effect which is exert through different mechanisms. Thereby, these seed varieties can be promoted as nutraceuticals with considerable antioxidant capacity. Cowpea is a popular pulse among Sri Lankan population which is widely cultivated in rural areas. Hence, promoting cowpea as a nutraceutical among rural area will be an effective strategy to ensure a healthy life of rural population preventing many diseases.

Conclusions and Recommendations

The present study revealed that seeds of four common local varieties of *V. unguiculata* exert the anti-oxidant potency in different mechanisms. In addition, it was observed that different secondary metabolites are mediated to exert different mechanisms. The present study suggest that all four varieties possess considerable antioxidant potency reflecting their value as nutraceuticals. Therefore, routine consumption of Sri Lankan varieties of cowpea is recommended to ensure a health life.

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EVALUATION OF ASPARTIC PROTEASE INHIBITORY ACTIVITY IN *Phaseolus vulgaris* (BEANS) GROWING IN SRI LANKA

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Introduction

Proteases are the enzymes that catalyze breakdown of proteins and peptides which are formed by covalently linked amino acids. Proteases can be categorized as aspartic, cysteine, serine, threonine and metallo proteases according to the amino acid residue available in the active site. Proteases are vital chemical compounds in all organisms which are involved in different physiological processes important for growth and development [1].

Among them aspartic proteases are acidic compounds and classified into 14 different subfamilies depend upon the amino acid sequence. They have been reported to involve in a wide range of physiological functions in organisms including digestion of nutrients (chymosin, pepsin A) and control of blood pressure (renin). Apart from that they are also mediated in metastasis of breast cancer (cathepsin D), pollen pistil interactions (cardosin A), the degradation of hemoglobin by parasites (plasmepsins) and maturation of HIV proteins (retropepsin). Hence, aspartic proteases are involved in pathogenesis of a certain diseases in humans such as hypertension, AIDS, malaria, Alzheimer's disease, and adult T cell leukemia [2].

Thereby, recently aspartic protease inhibitors have been identified as therapeutic strategy against the pathological conditions induced due to the activity of aspartic proteases. Certain inhibitors have been proved as anticarcinogenic, antiviral and antibacterial agents. They bind to the enzyme active site and reduce the enzymatic activity through preventing binding to the substrate. Natural aspartic protease inhibitors have been discovered in plants such as legumes, cereals, and tubers [2].

Although scientists in different countries have been largely studied different protease inhibitors in plants, such studies have not been carried out on the plants growing in Sri Lanka. Hence, the present study is designed to evaluate the aspartic proteases inhibitory activity in seeds of a local breed of *Phaseolus vulgaris* (beans) known as Gannoruwa Bil. The seed sample was screened for pepsin inhibitory

activity followed by assessment of the effect of different environmental conditions on the pepsin inhibitory activity.

Materials and Methods

Extraction of protease inhibitors from plants

The mature seeds of local breed of *Phaseolus vulgaris* known as Gannoruwa Bil were collected from the Field Crops Research and Development Institute of Sri Lanka (FCRDISL). Seeds were milled using an electrical blender and homogenized using a motor and pestle to prepare 20% aqueous extract. After centrifugation the supernatant was diluted to prepare a concentration series of the extract (10%, 5%, 2.5%, and 1.25%) [3].

Determination of the pepsin inhibitory activity of the seed extracts

The pepsin inhibitory activity was evaluated in each concentration using hemoglobin as the substrate. Each experiment was performed in triplicates at 4 °C. The seed extracts were mixed with phosphate buffer and pepsin, while the negative control was prepared by adding phosphate buffer to pepsin in the absence of seed extract. Phosphate buffer was mixed only with seed extracts to prepare the experimental blank. These mixtures were incubated for 15 minutes at 37 °C. Then hemoglobin was added to each mixture and incubated at 37 °C for 30 minutes, following addition of trichloroacetic acid. Each sample was then centrifuged and the absorbance of the supernatant was measured at 280 nm against the blank. Percentage inhibitory activity was calculated and the concentration which exert the maximum activity was selected for characterization of pepsin inhibitory activity in Gannoruwa Bil seeds [4].

Determination of the effect of temperature on pepsin inhibitory activity

The 10% seed extract was mixed with pepsin and incubated at different temperatures (0 °C, 20 °C, 37 °C, 60 °C, 80 °C, 100 °C) for 30 minutes. The negative control and the blank were prepared accordingly. Afterwards pepsin inhibitory activity was measured as mentioned above [5].

Determination of the effect of pH on pepsin inhibitory activity

The 10% seed extract was mixed with pepsin and incubated at 37 °C with phosphate buffers with varying pH (5.8, 6.4, 7.0, 7.4, 8.0). The negative control and the blank were prepared accordingly. Following incubation for 30 minutes pepsin inhibitory activity was measured as described above [5].

Determination of the effect of metal ions on pepsin inhibitory activity

The pepsin inhibitory assay was carried out after incubation of seed extract (10%) with different metal ion solutions including BaCl₂, Ferric chloride, Zinc acetate, Copper acetate, NaCl [5].

All the assays were performed in triplicates. The results were statistically analyzed by SPSS 20.

Results and Discussion

The pepsin inhibitory activity of the seed extracts

Among the concentrations tested, the highest percentage pepsin inhibitory activity was observed in the 10% concentration of the seed extract. The lowest inhibitory activity was exerted by the 1.25% seed extract. Therefore, the 10% concentration of seed extract was selected for further studies (Table 1).

Table 1. Percentage enzyme activity in different seed extract concentrations

Plant extraction concentration %	Percentage Pepsin inhibition activity (%)±SD
20%	36.19±0.03
10%	48.66±0.03
5%	40.60±0.02
2.5%	36.89±0.02
1.25%	35.52±0.01

The effect of temperature on pepsin inhibitory activity

The highest percentage of inhibitory activity was demonstrated at 37 °C, which was not significantly ($p>0.05$) different from the activity of the control. Although the enzyme inhibitory activity was decreased gradually with increased temperature, the inhibitors present in seed sample retained inhibitory activity to certain extent even at high temperatures as 80 and 100 °C (Figure 1).

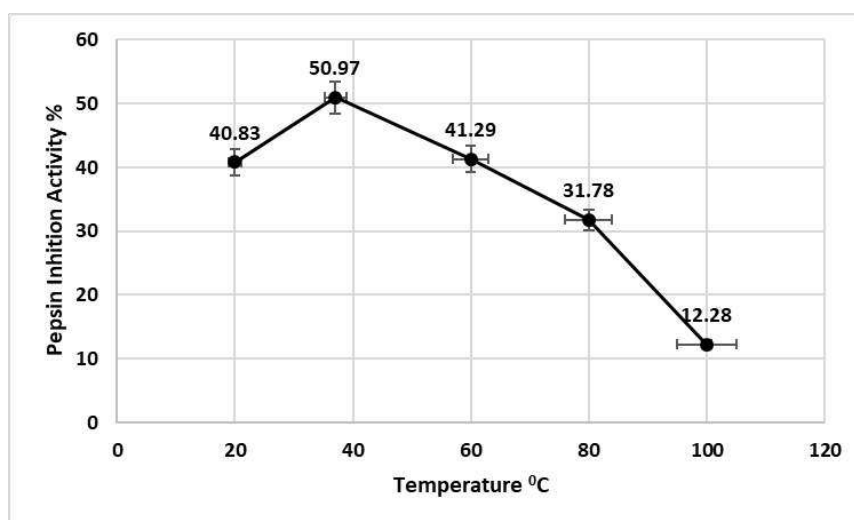


Figure 1. Percentage enzyme inhibition activity of Bill bean (10%) at different temperatures

The effect of pH on pepsin inhibitory activity

The lowest enzyme inhibition activity was exerted at pH 5.8. Comparatively, enzyme inhibition activity was higher in other pH values. A sharp increase in the inhibitory activity was observed at pH 7 (Figure 2), which was not significantly ($p>0.05$) different from the activity of the control.

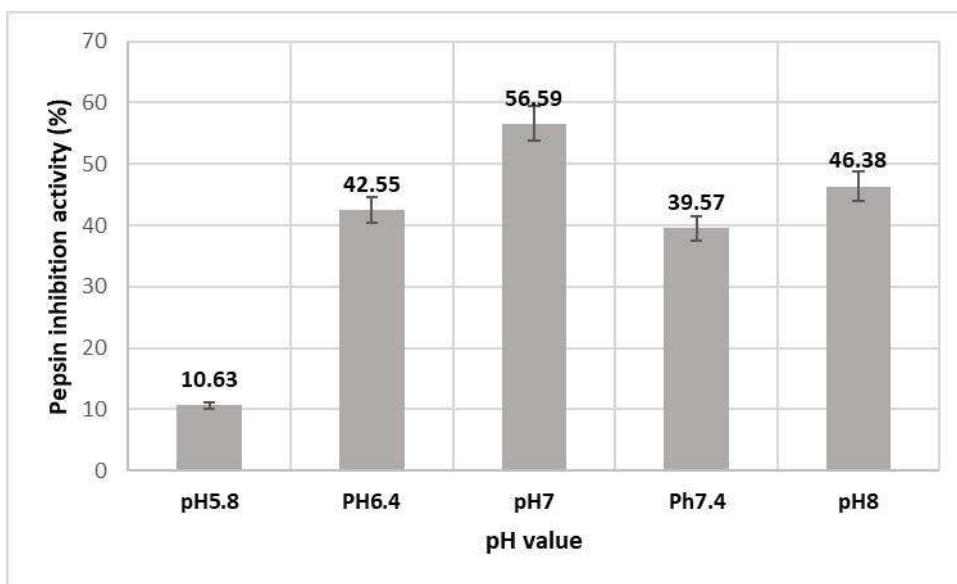


Figure 2. Percentage enzyme inhibition activity of seed extract (10%) at different pH

The effect of metal ions on pepsin inhibitory activity

The highest percentage inhibition activity was performed in the presence of Na⁺ ions. The metal ions, Ba²⁺ and Cu²⁺ also showed a positive effect on the enzyme inhibition activity. However, the presence of ferric ions reduced the inhibitory activity moderately ($p>0.05$), while Zn²⁺ ions reduced the enzymatic inhibition significantly ($p<0.05$) compared to the control. (Figure 3).

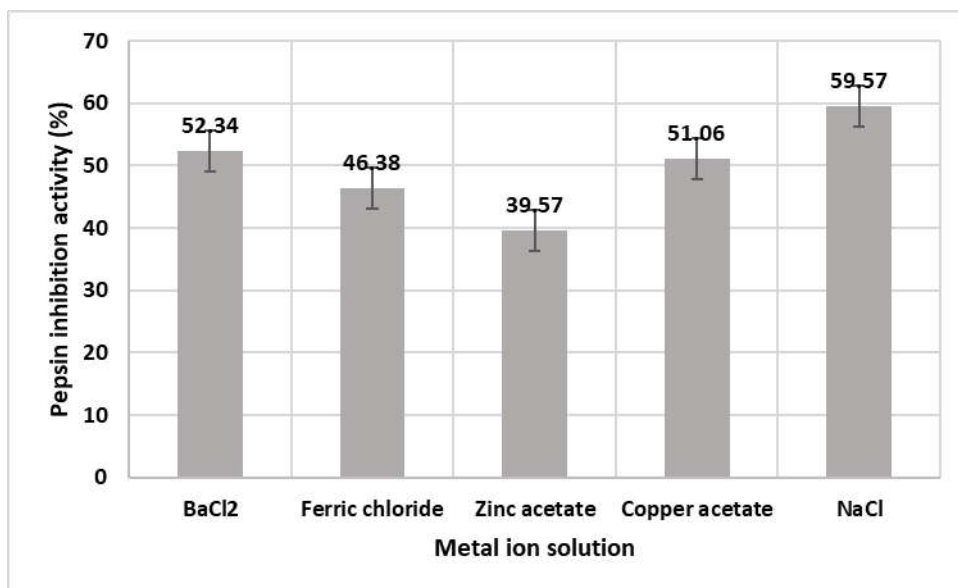


Figure 3. Percentage enzyme inhibition activity of Bill bean (10%) in the presence of different metal ions

Conclusions and Recommendations

The results of the present study suggest that the local varieties of *Phaseolus vulgaris* (beans) known as Gannoruwa Bil contain a considerable amount of pepsin inhibitors. Therefore, they can be used as sources for the discovery of active pepsin inhibitors for therapeutic applications. The characterization data will be useful in further experiments that will be carried out to purify the active compounds.

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URBAN CONSUMERS' PERCEPTION AND BUYING BEHAVIOR TOWARD VIRGINE AND NORMAL COCONUT OILS

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Introduction

Vegetable oils are an important part of our everyday diet. The demand for vegetable oils has increased with the increasing population and the discovery of new uses of them. The estimated average global per capita consumption of vegetable oils during the 2001–2011 period was 10.71 kg and it is 1.24 kg higher than the same value for the previous decade [1]. In Sri Lanka, coconut oil is the main type of vegetable oil used in cooking. Around 15–20% of the annual fresh coconut produce is used for coconut oil production (mainly copra oil), which primarily caters to the domestic market [1]. Further, virgin coconut oil production is emerging as a promising industry in the country due to increasingly recognized health benefits and global demand [1]. Virgin coconut oil and normal coconut oil are two oil types that seem similar. Thus, consumers are confused and find it difficult to differentiate between them. Furthermore, most of the urban consumers are uninformed about these two and are unaware of the differences. With the busy lifestyle, they mainly focus on buying coconut oil for making food without considering the type of coconut oil. Even though they look and sound similar, they are diametrically opposed [2]. Normal coconut oil has been found to be adulterated, either accidentally or purposely. Adulteration of food has several negative health consequences in addition to lowering its quality. At present, people purchase coconut oil without considering this matter. Generally, coconut oil is sold both in bottled and un-bottled formats, where bottled ones are packaged under various brand names. Abundantly, adulterated coconut oil causes human heart diseases. Besides these reputed brands also found to be adulterated with ordinary palm oil or other cheap oils [3]. Heart Disease Deaths in Sri Lanka reached 28,777 or 22.64% of total deaths. The age adjusted Death Rate is 123.79 per 100,000 population ranks Sri Lanka 94 in the world [4]. Therefore, the main aim of this study was to assess the urban consumers' perception toward coconut oil and examine the relationship between socio-economic factors and urban consumers' buying behavior toward coconut oil.

Materials and Methods

This study was conducted in the three of the main urban DS divisions (Colombo, Homagama, and Maharagama) in the Colombo District in Sri Lanka. The main three DS divisions were randomly selected for the study. From these three DS divisions, 300 consumers were selected as the sample of the study using a simple random sampling technique. Primary data was collected through a self-

administered questionnaire survey. Data collection was carried out from August to December 2020. Data analysis was done using importance-performance analysis, descriptive analysis, and chi-square test as the analysis methods. Importance performance analysis was used to examine the consumer perception toward the normal and virgin coconut oil [5]. Chi-square was used for analyzing the relationship between factors and consumer buying behavior on coconut oils. SPSS software was used in data analysis.

These Hypotheses were used in the study.

H₁- There is no significant relationship between gender and consumer buying behavior on coconut oils.

H₂- There is no significant relationship between age and consumer buying behavior on coconut oils.

H₃- There is no significant relationship between race and consumer buying behavior on coconut oils.

H₄- There is no significant relationship between education level and consumer buying behavior on coconut oils.

H₅- There is no significant relationship between income level and consumer buying behavior on coconut oils.

H₆ – There is no significant association between the heart disease problems of respondents and consumer buying behavior on coconut oils.

Results and Discussion

Consumer perception on virgin coconut oil

Calculation of conformity level between the level of performance and level of importance is shown below in (Figure 1). The average of importance is greater than the average of the performance of virgin coconut oil (2.89 for performance <3.49 for importance). It means that there is a gap between quality and expectation. The average value of the gap is 0.6. Based on Importance Performance analysis mapping (Figure 1), it can be analyzed that the perception of virgin coconut oil is in I, II, and IV consciousness with the following results;

1. Quadrant I (Concentrate Here)

Attributes included in Quadrant I are quality, appearance, and information about product ingredients of virgin coconut oil. The inclusion of these attributes is greatly influenced the consumer's desire to purchase virgin coconut oil. Factors located in this quadrant are considered as Important and/or Expected Factors by consumers.

2. Quadrant II (Keep up the good work)

Items included in quadrant II are product taste, price, availability, and promotion of virgin coconut oil. Factors located in this quadrant are considered important and expected as a supporting factor for customer perception.

3. Quadrant IV (Possible overkill)

Packaging of virgin coconut oil attribute position in Quadrant IV. This attribute is of low importance for consumer perception of virgin coconut oil.

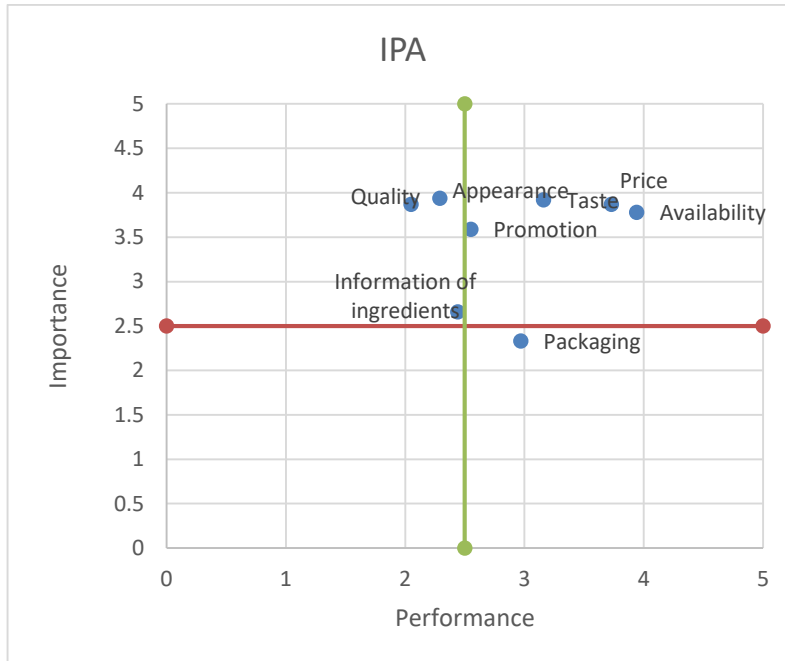


Figure 1. IPA mapping of virgin coconut oil

Consumer perception towards normal coconut oil

The results show that the average performance compared to the average of importance is greater (2.98 for performance < 3.55 for importance), which means that there is a gap between quality and expectation value. It is a gap of 0.57. Based on IPA mapping (Figure 2), it can be analyzed that the attributes of normal coconut oil are in I, and II consciousness with the following results;

1. Quadrant I (Concentrate Here)

Product price, promotion, and availability of normal coconut oil are considered as important and/or expected factors by consumers. The inclusion of those attributes greatly influences the consumer's desire to purchase regular normal coconut oil.

2. Quadrant II (Keep up the good work)

Items included in quadrant II are product taste, quality, appearance, information about the product ingredients, and packaging of normal coconut oil. Factors located in this quadrant are considered important and expected as a supporting factor for customer perception.

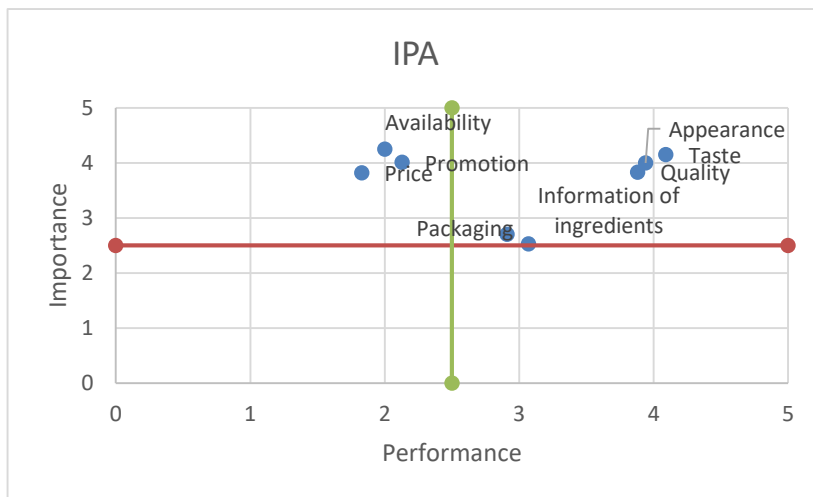


Figure 2. IPA Mapping of normal coconut oil

The relationship between socio-economic factors and urban consumers' buying behavior on coconut oil

The majority of consumers had engaged in buying coconut oil for cooking purposes. Among them, 54% of respondents were familiar with buying normal coconut oil and only 10% of respondents were familiar with buying virgin coconut oil. About 30% of consumers had purchased palm oil (vegetable oil) and few respondents were familiar with other types of edible vegetable oil such as Sesame oil, olive oil, etc. Based on a study in Ghana, Nondzor (2015) reported that the majority of respondents had engaged in buying palm oil. However, according to a study in Gampaha and Anuradhapura districts in Sri Lanka, Amarathunga (2000) mentioned that majority of respondents were engaged in buying coconut oil. The age, race, educational level, occupation, and income level of the urban consumers showed relationships with the buying behavior of coconut oil. However, the gender factor was not shown a relationship with the buying behavior of coconut oil (Table 1). But Bhuvaneswari, (2015) has mentioned in the study in Coimbatore city, India that only gender was shown the relationship with the buying behavior on coconut oil. However, the consumers' buying behavior dissection depends on the consumers' age, race, educational level, occupation, and income level due to the price differences and their different perception ways.

Table 1. Association between socioeconomic factors and buying behavior on edible vegetable oil

Factor	Buying behavior on edible vegetable oil				Asymp. sig	H ₀
	Normal coconut oil	Virgin coconut oil	Both	Other		
	Frequency	Frequency	Frequency	Frequency		
Gender					0.056	Not rejected
Male	72	32	28	00		
Female	128	56	40	12		
Age					0.000	rejected
18-30	16	32	20	08		
31-40	76	32	16	00		
41-60	84	20	32	00		
60<	24	04	00	04		
Race					0.003	rejected
Sinhala	176	84	52	12		
Muslim	20	04	16	00		
Berger	04	00	00	00		
Educational Level					0.001	rejected
Up to O/L	92	44	44	08		
Up to A/L	64	32	24	04		
Higher education	44	12	00	00		
Occupation					0.000	rejected
Government	40	44	08	00		
Semi-government	08	08	12	00		
Private	100	16	28	08		
Self-employed	36	08	16	04		
Retired	16	08	00	00		
Unemployed	00	04	004	00		
Income Level (LKR)					0.000	rejected
<25000	00	04	00	00		
25000-35000	08	00	00	00		
36000-45000	44	08	24	00		
46000-65000	48	32	20	04		
66000-85000	32	20	12	08		
86000-100000	52	20	12	00		
100000<	16	04	00	00		

The relationship between health factors and urban consumers' buying behavior on coconut oil

The Pearson chi-square significant value was 0.000 which is less than 0.05. Therefore, there is a significant relationship between the health factor and buying behavior on coconut oils. Based on that, consumers have abundantly suffered from heart diseases under the health factor category. Diseases which are mostly familiar with purchasing normal coconut oil. But the consumers who are familiar

with purchasing virgin coconut oil are considerably low levels of suffering heart diseases [3]

Table 2. The relationship between health factors and urban consumers' buying behavior on coconut oil

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	129.343 ^a	9	.000
Likelihood Ratio	164.867	9	.000
Linear-by-Linear Association	20.089	1	.000
N of Valid Cases	300		

Conclusions and Recommendations

The majority of consumers have buying behavior toward the normal coconut oil. However, only 10% of respondents have purchased on virgin coconut oil. Urban consumers' socio-economic factors age, race, educational level, occupation, and income level are shown significantly relationship with the consumer buying behavior on coconut oils. Based on the result, the consumers have different perception levels for the normal and virgin coconut oil. For the virgin coconut oil, the respondents are given the main priority for the quality, appearance, and information about the product ingredients when purchasing. Moreover, the respondents are given the main priority for the price, availability, and promotion when purchasing normal coconut oil. There is a significant relationship between the respondents' health factors and buying behavior on coconut oils. However, the consumers have engaged in purchasing normal coconut oil from only focusing on price, availability, and promotion strategies.

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QUANTIFICATION OF TOTAL SUGAR CONTENT IN COMMONLY CONSUMED FOODS AND BEVERAGES IN SRI LANKA BASED ON WORLD HEALTH ORGANIZATION (WHO) NUTRIENT PROFILE MODEL FOR SOUTH EAST ASIA REGION (SEAR)

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Introduction

As a result of rapid urbanisation and changes in life style, non communicable Diseases (NCD) is in rise these days in our society [1]. It is identified that consumption of foods high in sugar as one of the major causes for diet related non communicable diseases such as diabetes. Therefore, World Health Organisation recommends adults and children to reduce consumption of free sugars to less than 10% of total energy intake [2]. Further more World Health Organisation developed a Nutrient Profile Model, which categorizes foods that are more likely to be constituents of a healthy diet. Nutrient Profile Model was developed for South-East Asia region with the objective of regulating marketing of foods and non-alcoholic beverages (FNAB) to children. Since there is not a data base which consists of sugar content of foods consumed commonly in Sri Lanka, it is important to quantify sugar content in foods to reduce obesogenic environment and to promote healthy diets.

Materials and Methods

Randomly collected food samples were categorized according to World Health Organization nutrient profile model for South East Asia Region. Total sugar content of food categories, juices (BEVa, n=14), milk and dairy based drinks (BEVb, n=29), cereal, grain, nut based beverages (BEVe, n=6), water based flavored drinks (BEVc, n=13), bread and ordinary bakery wares (BOB, n=29) curded dairy based desserts (CDD, n=16), cereals (CER, n=13), confectionary (CON, n=43), composite foods (CPF, n=12), sauces, dips and dressings (DRE, n=8), fine bakery wares (FBW, n=45), frozen dairy based desserts and edible ices (FDD, n=9), processed fruits and vegetables (PFV, n=12), potato cereal or starch and animal based savory snack foods (SSFa, n=24), processed nuts (SSFb, n=17) were analyzed by Lane and Eynon method. For that, to 10 g of homogenous sample which was dissolved in known volume of distilled water, 5 mL of potassium ferrocyanate and 5 mL of zinc acetate solution was added to clarify solution. Aqueous extract of sample was filtered with a fast filter paper. To 100 mL of filtrate, 15 mL of 1 molL⁻¹ HCl solution was added and allowed one-minute boiling. After cooling to room temperature, solution was neutralized by using 50% (m/V) NaOH solution, in the presence of phenolphthalein as an indicator. The solution was transferred to a 200mL volumetric flask and made up to mark by using

distilled water. By using sample solution, 10 mL of mixture of 1:1 of Fehling's A solution and Fehling's B solution which was placed over a hot flame allowing solution boil at a moderate rate without moving flask or altering flame, was titrated in the presence of 1% aqueous methylene blue solution until disappearance of blue colour leaving brick red colouration. The mixture of 1:1 of Fehling's A solution and Fehling's B solution was standardized by using standard dextrose solution. In house prepared control sample, which has known total sugar content, was analyzed to assure quality of test results.

Results and Discussion

The range of total sugar content (g/100 g \pm standard deviation) of food groups and threshold limits given in the model are mentioned in table 1.

Table1. Range of total sugar content of food groups and threshold limits given in SEAR model for each category

Food group	Range of sugar content (g/100g \pm standard deviation)	Threshold limit (g/100g)
Juices (beva)	7.4 \pm 0.02 - 15.4 \pm 0.02	6g/100g
Milk and dairy based drinks (bevb)	4.9 \pm 0.07- 19.8 \pm 0.02	not specified
Cereal, grain, nut based beverages (beve)	0 - 34.6 \pm 0.61	6g/100g
Water based flavored drinks (bevc)	0 – 20.1 \pm 0.03	2g/100g
Bread and ordinary bakery wares (BOB)	0 - 27.9 \pm 0.01	6g/100g
Curded dairy based desserts (CDD)	2.5 \pm 0.01 – 39.6 \pm 0.05	6g/100g
Cereals (CER)	0 – 25.8 \pm 0.02	9g/100g
Confectionary (CON)	0 – 96.9 \pm 0.38	6g/100g
Composite foods (CPF)	0 – 8.89 \pm 0.03	9g/100g
Sauces, dips and dressings (DRE)	0 – 48.2 \pm 0.08	10g/100g
Fine bakery wares (FBW)	0 – 51.0 \pm 0.47	6g/100g
Frozen dairy based desserts and edible ices (FDD)	9.6 \pm 0.07 – 23.2 \pm 0.06	12g/100g
Processed fruits and vegetables (PFV)	0 – 70.0 \pm 0.01	not specified
Potato cereal or starch and animal based savory snack foods (ssfa)	0 – 34.9 \pm 0.02	not specified
Processed nuts (ssfb)	0 – 51.6 \pm 0.01	not specified

According to World Health Organisation nutrient profile model for SEAR, threshold limit for total sugars for frozen dairy based desserts and edible ices (FDD) is 12 g/100 g. Threshold limit for total sugars according to model, for sauces, dips and dressings (DRE) is 10 g/100 g and for composite foods (CPF) and cereals (CER) is 9 g/100 g. For confectionary (CON), fine bakery wares (FBW), bread and ordinary bakery wares (BOB), juices (BEVa), cereal, grain, nut based beverages (BEVe), and curded dairy based desserts (CDD), threshold limit for total sugars is

6 g/100 g. For water based flavored drinks (BEVc), threshold limit is 2 g/100 g. In the model threshold limit for total sugars has not specified for potato cereal or starch and animal based savory snack foods (SSFa), processed nuts (SSFb), milk and dairy based drinks (BEVb), and for processed fruits and vegetables (PFV).

In food category juices (BEVa) which consist of 100% fruit and vegetable juices prepared from direct extraction or reconstituted from concentrates all samples exceed threshold limit for total sugars. Among the categories, from the tested samples, 100% of juices (BEVa), 77% of water based flavored drinks (BEVc), 67% of cereal, grain, nut based beverages (BEVe), 94% of curded dairy based desserts (CDD), 85% of cereals (CER), 26% of bread and ordinary bakery wares (BOB), 77% of confectionary (CON), 25% of sauces, dips and dressings (DRE), 91% of fine bakery wares (FBW) and 89% of frozen dairy based desserts and edible ices (FDD) exceed the threshold limits given for each category in SEAR model for total sugars. But all test items belong to food category of composite foods (CPF) adhere to threshold limits given in model for total sugars.

Conclusions and Recommendations

Based on threshold limits for total sugars provided in model, it is important to implement recommendations to prohibit marketing of foods and beverages high in total sugars to children to promote healthy diets. Furthermore, it is recommended to use threshold limits given in model to develop bench marks for foods sold in school cafeterias and to educate children and adults and to improve nutrition literacy. Since most of the samples tested exceed threshold limits, it is vital to force manufacturers to reformulate food products to reduce total sugar content to be inline with threshold limits given in the model for food groups to minimize prevalence of diet related non communicable diseases.

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GROWTH AND YIELD PERFORMANCES OF KANGKONG (*Ipomea aquatica*) IN RESPONSE TO DIFFERENT ANIMAL MANURE AND UREA FERTILIZER

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Introduction

Kangkong (*Ipomea aquatica*) a hollow stemmed leafy vegetable which is grown in Sri Lanka belongs to the family Convolvulaceae. They are usually found near water bodies, the edge of paddy fields, the marsh area or near a tank. There were two types of green Kankong namely 'Local' and 'Thai Kangkong' which are cultivated at a commercial scale in Sri Lanka. Kangkong is ready to harvest in 30 to 45 days after sowing or transplanting. The Sri Lankan government has banned the imports of fertilizers and agrochemicals on May 6, 2021. As a result, organic farming has become more popular throughout the country. Organic matter applied to the soil is a beneficial method for improving soil fertility as it recycles and mineralizes nutrients and reclaims degraded soil [1].

Organic materials increase soil nutritional status while impacting other soil qualities like aeration, water holding capacity, and particle aggregation. Nitrogen has beneficial effects on the plant vegetative stage. Nitrogen is one of the critical nutrients required by plants in large amounts, which is an important growth variable influencing the production and quality of crops. In Sri Lanka, urea is mostly used as the source of nitrogen, and split application of this fertilizer is commonly practised. Nowadays low soil fertility is in a large part of the lands in Sri Lanka; mainly because of the high yielding varieties, the application of excessive inorganic fertilizers and inadequate nutrient replacement; besides, over-fertilization results in the accumulation and leaching of nitrate residuals that directly contribute to the pollution of groundwater. This organic matter added to the soil is a favourable remedy to enhance the soil fertility and reclamation of the already degraded soil. Cattle manure, poultry manure and goat manure are the extensively used organic manure to fertilize leafy vegetables. Farmers use organic manure but do not manage them properly. Therefore, the efficiency of utilization of manure is very low. Considering the above factors, the present experiment was undertaken to study the response of different animal manure on the growth and yield of kangkong.

Materials and Methods

The experiment was conducted in an open field at Ragama (WL3) during the Maha season in 2019. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The treatments were urea (T0), cattle manure (T1), goat manure (T2), pig manure (T3), poultry manure (T4) and Zero nutrients (Blank test/ control) (T5). In the control, treatment urea was applied as the basal dressing 90 kg/ha which was recommended by the Department of Agriculture (DOA), in the blank test only the soil was there and all other treatments were having 2 months old wet animal manure. There were 15 plots and each plot dimension were 1 m x 2 m and 25 seedlings were planted in each plot at a spacing of 30 cm between rows and 20 cm between plants. The nitrogen percentage of animal dungs were determined by the Micro-Kjeldahl method and then according to the DOA recommendation, the amount needed to incorporate soil was calculated separately (Table 1). The calculation was done as follows;

The amount needed to incorporate in the soil (Kg/ha) =

$$\frac{\text{N\% in Urea} \times \text{DOA recommended nitrogen rate (Kg/ha)}}{\text{N\% in the animal dung sample}}$$

Table 1. The calculated amount of animal dung applies in grams per plot

Treatment	N % of the material	KG/HA	G/M2	G/PLOT
Urea (T0)	46	90	9	18
Cattle manure (T1)	1.6	2587	258	516
Goat manure (T2)	1.8	2300	230	460
Pig manure (T3)	2.8	1478	147	294
Poultry manure (T4)	2.3	1800	180	360

Animal dung was applied two weeks before transplanting as the basal dressing. Data were collected 2 weeks after transplanting (WAT) and at weekly intervals. The number of leaves, plant height, leaf length, leaf width, marketable yield and dry mass of economic yield were collected as parameters. Data were analysed using the analysis of variance (ANOVA) procedure by SAS and mean separation was done using Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

Results and Discussion

The results were recorded as the mean value \pm standard deviation. The highest mean number of leaves were recorded in T4 (12 ± 2 leaves) and the least number was in T3 (8 ± 1 leaves). But there was a significant difference ($p < 0.05$) between urea (T0) and poultry manure (T4). The highest mean plant height was in T4 (21.9 ± 4.1 cm) whereas the lowest mean height was in T5 (17.2 ± 3.4 cm). There was a significant difference ($p < 0.05$) in plant height between T0 and T4. The longest mean leaf blade length was in T4 (7.1 ± 2.4 cm) and the shortest leaf blade was in T5 (5.5 ± 0.2 cm). Then the highest mean leaf width was in T4 (3.1 ± 0.3 cm) while the lowest width was in T5 (2.4 ± 0.6 cm). The highest mean leaf petiole length was in T4 (4.1 ± 0.2 cm) whereas the least was in T5 (3.2 ± 0.1 cm). The longest mean internodal length was in T4 (2.8 ± 0.6 cm) whereas the shortest internodal length was in T5 (2 ± 0.2 cm). The mean number of leaves, plant height, leaf blade length, leaf width and leaf petiole length of poultry manure (T4) and urea (T0) were showed a significant difference ($p < 0.05$) and it was because the poultry manure contained more plant nutrients including phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) than all other organic manures even though pig manure recorded the highest nitrogen percentage in this study [2].

Table 2. Average and total fresh yield, and total dry yield of kangkong grown under different fertilizer regimes

Treatments	Avg yield (g)/plot/harvest	Total yield (g)/plot	Total dry yield (g)/plot
Urea (T0)	151 ^b	1510 ^b	377.5 ^b
Cattle Manure (T1)	165 ^a	1630 ^a	410.2 ^a
Goat Manure (T2)	154 ^b	1540 ^b	380.4 ^b
Pig Manure (T3)	145 ^c	1452 ^c	362.5 ^c
Poultry manure (T4)	163 ^a	1651 ^a	412.5 ^a
Control (T5)	130 ^d	1325 ^d	325.4 ^d

*Values within a column followed by a common letter are not significantly different at $P=0.05$, according to DMRT

The highest total yield per plot (1651 ± 138 g) was in T4 whereas the lowest (1325 ± 180 g) was in T5. The yield per plot of T4 and T0 was significantly different ($p < 0.05$) whereas cattle and poultry manure did not show a significant difference ($p < 0.05$) respective to the total yield per plot. Cattle and goat manure showed a significant difference ($p < 0.05$) respective to the total yield per plot. Cattle manure reported a higher total yield per plot than goat manure; it was because cattle manure decomposed faster than goat manure; whereas it has low nitrogen concentrations and a high C/N ratio [3]. The highest total dry yield per plot (412.4 ± 26 g) was in T4 whereas the lowest (325.4 ± 32 g) was in T5. The dry yield per plot of T4 and T0 were significantly different ($p < 0.05$) whereas cattle and poultry manure did not show a significant difference ($p < 0.05$) respective to the

total dry yield per plot. Poultry manure is usually a free-flowing material made up of bigger particles that contain various nutrients required by plants after being removed from the shed plants [4]. The soil could be enriched with the application of organic material which tends to decompose and release relatively large amounts of nitrogen into the soil before planting a crop to boost yield [5]. Industrial fertilizers do not possess good characteristics of aggregating the soil particles; as a result, cultivable soil gets depleted and degraded and therefore the best remedy was the organic matter in cooperation [5]. It also concluded that poultry manure contains various nutrients like nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) that were diluted solutions similar to that found in the soil in the form of moisture films around soil particles [2]. Similar to the present study; it was observed that on yield and quality of leafy vegetables grown with organic fertilizers showed better growth than vegetables grown with organic fertilizers [5]. Considering the results, it was obvious that it can apply animal manure to compensate for the nitrogen requirement even though large quantities are needed.

Conclusions and Recommendations

The growth parameters such as the mean number of leaves, plant height, leaf blade length, leaf width and leaf petiole length whereas the average yield per plot, total yield per plot and total dry yield per plot reported in poultry manure (T4) were significantly different ($p < 0.05$) with urea applied treatment (T0).

Hence it was obvious that the growth and yield performances of Kangkong were affected significantly by poultry manure. Since animal manure contains a low nitrogen percentage it is needed to add a large amount of animal manure when compared with inorganic fertilizer.

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EVALUATION OF CULTURE CONDITIONS AND CHEMICAL COMPOSITION OF *AZOLLA PINNATA*

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Introduction

Azolla is an aquatic free floating fern which can be grown in diverse fresh water systems like ditches, ponds, canals and paddy field that have shown rapid growth in natural environment. It is commonly found in tropics, subtropics, and warm temperate regions of Asia, America and Africa. The fern has natural symbiotic relationship with blue green algae *Anabaena*, which is responsible for fix high amount of atmospheric nitrogen in its leaves [1], [2].

Nowadays, livestock industry including poultry and cattle is one of the most profitable agriculture-based businesses in Sri Lanka. Large numbers of new farms are established throughout the country. However higher feed cost is a big challenge in Sri Lankan livestock industry. Therefore, many farmers are moved to alternative cheap nutrition sources.

Azolla is economical and easy to be cultivated which can be used as an ideal feed for livestock. Feeding *Azolla* has shown increased egg production in layers and improvement in weight of broiler chicken [1]. Even though it is used as a fresh feed, it can be used as one of the feed ingredient in animal feed formulation. Still, only certain Asian countries are successfully utilizing this plant as live stock feed such as China, India, Bangladesh and Vietnam. However, utilization of *Azolla* as a substitute feed in animal feeding is an emerging concept in Sri Lanka and also the production of *Azolla* is limited to certain small scale producers.

Azolla is not only used as animal feed source but also used as source of nitrogen for organic fertilizer. *A. pinnata* can be added to rice field as an organic fertilizer as in fresh form or composted. Despite the known benefits of *Azolla*, a few researches have been done in Sri Lanka. There was lack of studies on nutritional value of *Azolla* in Sri Lanka. This study will offer benefit, especially about the culture and nutrition value of *Azolla*, thus farmers and raisers will gain awareness about low-cost production of *Azolla* that leads to improve the livestock production in an economic way.

Materials and Methods

Culture and management

The study was carried out for two months, during March to May 2021 at backyard of house of the researcher in Jaffna district, Sri Lanka. A temporary small pond was created with dimensions of 3m × 2m × 0.3m in a shady region and covered with black plastic sheet that properly secured. To initiate the *Azolla* culture, little amount of sieved soil mixed with cow dung was added over the plastic sheet then fresh water was added and fresh 100 g of *Azolla* was inoculated. The pond was allowed to completely cover with *Azolla*, and then *Azolla* was partially harvested. Cow dung was added in two weeks interval also around 25% water was replaced once in 10 days in order to prevent the nitrogen build up in bed. During study period, some important water quality parameters of the experimental pond were monitored weekly by using a portable multi parameter.

Chemical analysis

Harvested *Azolla* were cleaned and initial fresh weight was measured, then *Azolla* was sun dried in shady area for three days until constant weight, crispy and green colour retained. The collected dried *Azolla* was packed in polythene bags for further use. The proximate composition was estimated according to standard AOAC method. The sun dried *Azolla* sample was analyzed for its dry matter content by using an oven at 105 °C until constant weight, ash content and organic matter were calculated by incinerating it in muffle furnace at 550°C until constant weight. The crude protein was estimated by Kjeldhal method and ether extract determined by Soxhlet system using petroleum ether. Gross energy value was estimated by E2K Bomb calorimeter.

Statistical analysis

All data generated were analyzed using descriptive statistics using R version 4.0.3. Statistical values that were calculated include mean and standard deviation.

Results and Discussion

Azolla was formed a dense mat on water surface and first crop was ready in after 7-10 days. It took 3-4 days to cover the water surface after removal of 1/3 portion of *Azolla*. The diameter of plant ranges from 1.8 to 2.3 cm.

Table 1. Observed water quality parameters of *Azolla* culture pond

Parameters	Observed value
DO (mg L ⁻¹)	5.21 - 5.79
Water temperature (°C)	25.37 – 27.95
Salinity (ppt)	0.4
Specific conductivity (µS/cm)	724.9 - 730.6
pH	6.5 – 7.67

The observed air temperature during study period was 29.8–32.4 °C. The water temperature was found to be 25.37–27.95 °C in the present study. According to present study the recorded water temperature and pH value (Table 1) were very close to the recommendation of [2] and who mentioned that high biomass and chlorophyll production of *Azolla* can be found at pH between 6 and 7. The dissolve oxygen of *Azolla* pond in present study was shown a positive result (Table 1.) that indicating the good quality of water in pond. The recorded water quality parameters were highly favorable for *Azolla* culture which provided high yield. The chemical composition percentage of *Azolla pinnata* is explained in Table 2. by dry matter(DM) basis.

Table 2. Chemical composition of sun dried *Azolla pinnata*

Chemical composition	Percentage (% DM)
Dry matter	89.36 ± 0.59
Crude Protein	22.71 ± 0.92
Ash	21.30 ± 0.71
Ether extract	2.62 ± 0.86
Gross energy (kcal g-1)	3.053 ± 0.01

Data represented as mean ± SD (n=3).

The estimated moisture content change in fresh *Azolla* while sun dried was 94.42%. The chemical analysis results of sun dried *Azolla* reveal that the dry matter content of sun dried *Azolla* was 89.36% (Table 2). This results is in close line with the results of Balaji *et al.* [3] Basak *et al.* [4]. The total ash percentage of sun dried *Azolla* was 21.30%. This result shows slightly higher value than the results obtained by Khursheed *et al.* [1], Balaji *et al.* [3] and Basak *et al.* [4]. Crude protein percentage of sun dried *Azolla* in this study was 22.71%, these findings are almost similar to the results obtained by Khursheed *et al.* [1], Balaji *et al.* [3] Basak *et al.* [4] and Khare *et al.* [5]. The high amount of protein content indicates that, *Azolla* is one of the cheapest plant protein sources which can be used as feed for cattle, poultry for a certain amount with consideration

of other nutrient factors. The ether extract of sun dried *Azolla* in the present study was 2.62% which was in close line with results obtained by Khursheed *et al.* [1], Balaji *et al.* [3] and Basak *et al.* [4]. Gross energy of sun dried *Azolla* was 3.053 kcal g⁻¹ and almost similar results were obtained by Khare *et al.* [5].

The chemical composition of *Azolla* may be influenced by several factors such as environmental conditions, soil and nutritional condition, strains and variety, application of fertilization and several other factors. The present study indicates that *Azolla* contains high crude protein, high energy content and high mineral content in terms of high ash content. Concern and utilization of aquatic plants is increasing nowadays. *Azolla* is one of the best and cheapest substitute feed and feed ingredient for livestock. Government and Non-Government Organizations should encourage conducting research and promotion of *Azolla* culture.

Conclusions and Recommendations

The study has shown that *Azolla* is a nutritionally valuable aquatic plant. *Azolla* can be grown rapidly in tropical water. *Azolla* have high protein content which can be used as a replacement to other protein sources for animal feed. Hence *Azolla* can be produce in a simple, easier and low-cost way, the *Azolla* cultivation in Sri Lanka may also help to reduce the feed cost and create new employment opportunities. Future studies should be carried out to explain the complete nutritional profile of *Azolla* such as mineral composition, fatty acid and amino acid profile.

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EFFECT OF CHEMICAL TREATMENTS AND COLD STRATIFICATION ON DORMANCY BREAKING TO PROMOTE GERMINATION IN ISRAEL BLUE (*Vitis vinifera* L.) GRAPES VARIETY

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Introduction

Seed Dormancy is a process of blocking germination completely in viable seeds under favorable environmental conditions. It can protect seeds from hard environmental conditions and can disperse through a large extended area. In the process of seed germination, grape seeds show low germination rates, a long period for germination and it affects badly in grapes breeding programs due to seed dormancy. Seed germination percentage and seedling vigor can be increased with the application of chemicals like Gibberellic acid and controlling the temperature [1] by breaking the seed dormancy. Grape seeds can be easily germinated under cold stratification with the favored low temperatures [2]. This study aims to determine the effects of four pretreatments with an application of chemicals and cold stratification under different temperatures less than 10°C to increase the germination capability of grape seeds within a short period.

Materials and Methods

The research was conducted in the Jaffna University Research and training center. The study was carried out in a two-factor factorial Complete Randomized Design using four replicates. Each replicate had 14 seeds. Totally 1120 grape seeds were sown.

Plant material

Open-pollinated seeds of Israel blue (*Vitis vinifera* L.) were used for all treatments. Fully ripe berries were collected from grape farmers in the Jaffna district. Seeds were extracted, washed, checked for viability, and air-dried for 2 days.

Methodology

Seeds received pretreatments with chemicals before cold stratification. 1) 48 hours water soak (control), 2) 24 hours soak in 1000ppm H₂O₂ followed by 24 hours soak in water, 3) 24 hours soak in GA₃ followed by 24 hours soak in water, 4) 24 hours soak in Acetic acid followed by 24 hours soak in water [3].

Seed stratification

Five different temperatures less than 10°C were used in this study. Temperatures of, 4°C, 5°C, 6°C, 7°C, and 8°C were chosen, correspondingly. Because of low-temperature differences between 10°C and 5°C stratification for 21 days quickly break the grape seed dormancy.

Seed sowing

Pretreated stratified seeds were sown in media (1:1:1 in ratio sand: topsoil: farm manure). Seeds with media were placed in a growth chamber by organized in a completely randomized design with five replicates by providing the conditions of 30 °C for 16 light hours (12.5 lux of light intensity) and 25 °C for 8 dark hours with 65 % relative humidity which condition is the ideal to promote germination in grapes.

Data Analysis

The number of seeds germinated under different treatment combinations; time taken for germination were measured. Collected data were analyzed in SAS 9.1 version and DMRT mean separation was done to identify the suitable treatment combination.

Results and Discussion

The results were shown that germination was increased with a cold stratification temperature of 4 °C and 5 °C with the application of Gibberellic acid 1000 ppm concentration. 30% of grape seeds germination was observed under Gibberellic acid application with stratification temperatures of 4 °C and 35 % grape seeds germination from the application of Gibberellic acid with stratification temperature of 5 °C.

Table 1. The interaction between four chemical applications and five stratification temperatures, after 50 days of grape seed germination

GSI (Germination Speed Index)					
Treatments	Stratification Temperatures				
	4°C	5°C	6°C	7°C	8°C
Water	15%	12.5%	7.5%	12.5%	0%
Hydrogen peroxide	25%	17.5%	17.5%	17.5%	17.5%
Acetic acid	15%	32.5%	12.5%	22.5%	15%
Gibberellic acid	30%	35%	15%	12.5%	17.5%

The percentage of seeds that germinated was gradually raised as the stratification period progressed up to 21 days. The germination of grape seeds was significantly improved when they were stratified at a low temperature.

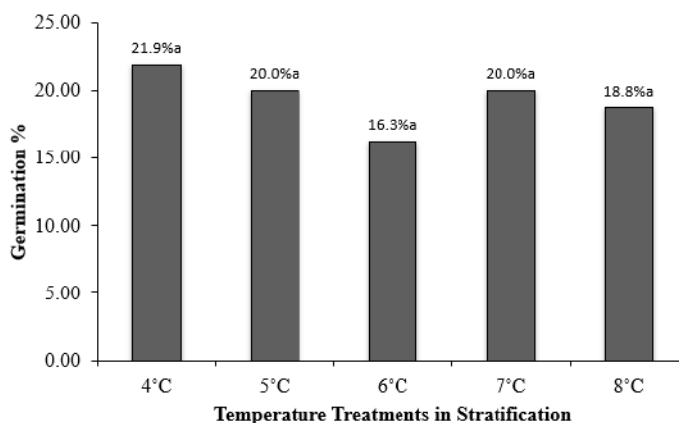


Figure 1. Grape seed Germination Percentage at alternate Stratification Temperatures. The same letters are indicated not significantly different by DMRT at $p=0.05$

There was a significant difference observed among the application of different chemicals as treatments when seeds were soaking (Figure 2). Grape seeds Soaking in acetic acid for 24 hours before cold stratification treatment results in a limited effect on seed germination (15%). Soaking grape seeds in Hydrogen peroxide solution had little effect on germination percentage (25.5%). Gibberellic acid especially at 1000 ppm, gave the best results concerning germination percentage (26.5%). It was found that the Gibberellic acid treatment promoted the germination of grape seeds (Darné, 1996). The application of Gibberellic acid will break the embryo dormancy of grape seeds and increase the Grape seed Germination percentage. Hydrogen peroxide stimulates dormancy breaking and can damage seed outer coat tissues.

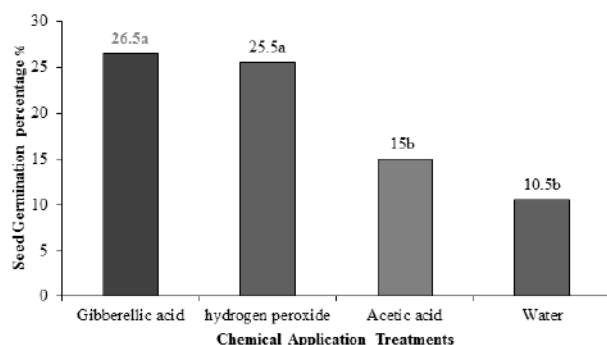


Figure 2. Grape seed Germination Percentage with the application of different chemical treatments when in Grape seed soaking. Different letters are indicated significantly different by DMRT at $p=0.05$

In general, grape seeds required 120 days for germination without pretreatments. [4,5]. But with the application of cold stratification treatments, it was taken around 27 days to germinate after grape seed soaking. Cold stratification is sensitive to induce breaking grape seed dormancy (Figure 3). When increasing the stratification period, will increase the ability of the grape seed to germinate.

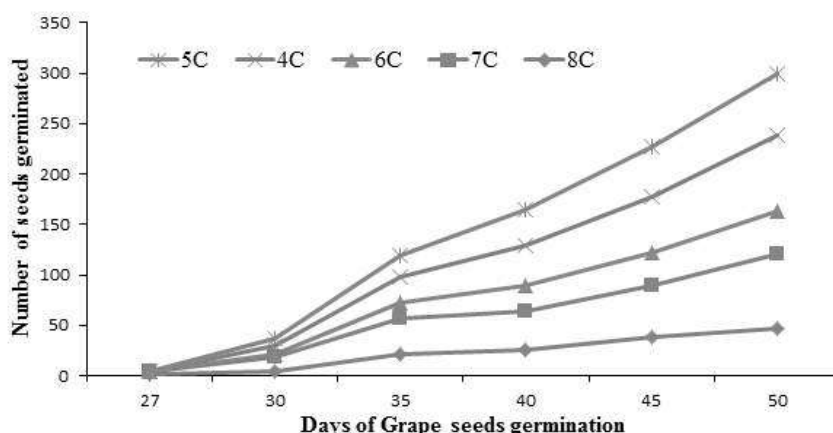


Figure 3. Mean grape seed germination with 27 days from seed soaking was observed with the effect of Stratification temperature treatments

The highest grape seed germination within a short period was obtained with the application of Gibberellic acid solution before sowing (Figure 4). Grape seed germination was taken place 12 days after the stratification period with the treatment of Gibberellic acid.

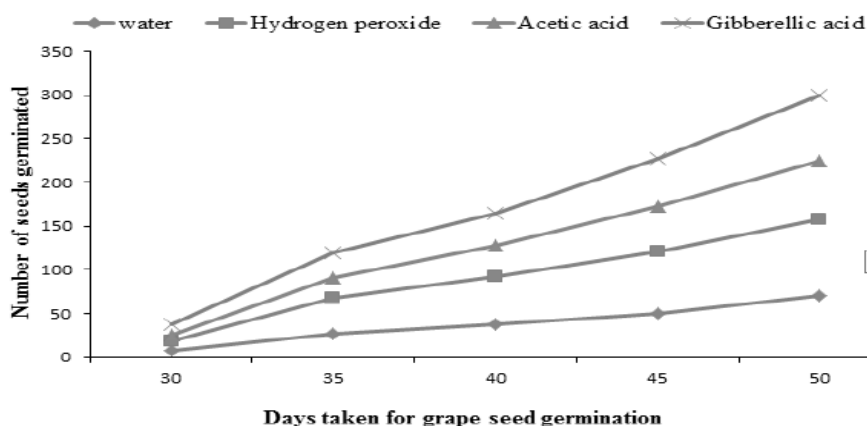


Figure 4. Mean grape seed germination with 27 days from seed soaking was observed with the effect of different chemical applications

Conclusion and Recommendations

From this study it can be concluded that Gibberellic acid application and cold stratification temperatures of 5 °C as good treatment combination to promote quick germination in grapes by breaking both embryo and seed coat dormancy.

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COMPARISON OF VERMIWASH WITH OTHER PREPARATIONS USING KITCHEN WASTES INTO A HYDROPONIC SYSTEM

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Introduction

Kitchen waste is known as the disposal garbage from the kitchen is the food that is not consumed. The causes of food waste or loss are numerous and occur throughout the food system, during production, processing, distribution, retail, and consumption. Agricultural industry has developed in many ways over the past two decades. Most of agricultural industries use fertilizer to do their productions. Fertilizers directly affect to the quantity and the quality of the agricultural production. In current society effective waste management such as vermiwash technique to actively manage the kitchen food waste and improve the production of vermiwash technique as a nutrient source for modern agri technology [1].

Vermiwash (VW) is a watery extract of vermiwash composts extracted in the presence of a wealthy population of earthworms. It contains several enzymes, plant growth hormones, and vitamins, along with micro and macronutrients. Vermiwash, if collected standard way is clear and transparent, pale yellow colored fluid [2].

This research work was conducted as comparison of vermiwash extracted from different species of earthworms and check their similarities and differences of different nutrient solutions. As well as check the probability of applying vermiwash into hydroponics.

Materials and Methods

Collection of food waste

Discarded food items were collected as the initial step to making sure that only organic matter was collected. As the next step, the organic waste was classified. Foods containing acid, portions of meat, discarded portions of vegetables and herbs were classified separately. Also, the waste from cooked food was collected separately.

Collection of earthworms

The earthworms were collected the required amount from the soil.

Experimental design for vermiwash extraction

When making the VW unit, used 20 of *Lampito mauritii* earth worms and first took a plastic basket and drilled a few small holes in its bottom because after the VW was created, they had to be filtered separately. Then drilled holes in the bottom of the basket and covered it with a cotton cheesecloth. This is because the VW is

filtered down to remove the large number of contaminants present in the falling VW. After the cotton layer, take a well-burned brick and break it into small pieces, and put it on top because it makes filtering even better. After a layer of burnt bricks, a thin layer of sand was applied on its top to streamline that further filtering. Then lay the sand layer on top of the soil and add the previously collected earthworms on top of it. Also, when collecting soil, care was taken to collect soil obtained from the areas where the earthworms lived. Then the created part was placed on top of another basket.

Chemical analysis

Chemical analysis of raw organic waste and final vermicompost of various wastes observed. Total Kjeldahl nitrogen was determined by the procedure. Total available phosphorus (TAP) was determined by the colorimetric method [3]. Total potassium, sodium, and calcium were determined by fire photometers[3]. PH value and electrical conductivity (EC) were determined

Hydroponic cultivation system

The next step was to investigate the probability of cultivating the obtained VW as a nutrient medium. Tomato seedlings have been substituted as planting material for this purpose. The VW was then mixed with water in the prescribed proportions. Also, as part of the control, the VW was diluted in water to the 1:10 ratio. That is the difference between these two feeding methods and getting the correct data in the VW.

Results and Discussion

VW cycle means collecting the organic food waste from the kitchen and then adding it to the VW bin with worms. Then prepare VW and, filter them & then add to the vegetable plants in hydroponics, also known as bio-fertilizer.

Chemical composition

Table 1. Chemical composition macro-micro nutrients of approached VW samples.

Parameters	Content
Nitrogen	120 ppm
Phosphorus	18500 ppm
Potassium	25± 2 ppm
Sodium	8±1 ppm
Calcium	3 ± 1 ppm
Copper	0.01 ± 0.001 ppm
Iron	0.06 ± 0.001 ppm
Magnesium	140 ppm
Manganese	0.57 ± 0.040 ppm
Zinc	0.01 ppm

There were contended macro & micronutrients according to table 1. The nitrogen content of VW is 0.012 - 0.005%. Phosphorus content is 1.85%. Potassium content is 25±2 ppm. So both VW macro & micronutrient compositions had nearly the same nutrient content.

pH values & electrical conductivity

Table 2. Comparison of pH & EC level of Albert solution compared reference VW sample & approached sample

	Albert solution	Substitute VW	Approached VW
pH level	5.4	7.39 – 7.5	7.7 – 8.62
EC	1.5 - 2.5 dS/m	1.2 dS/m	1.53 dS/m

According to table 2, the pH and electrical conductivity were 7.7 – 8.62 and 1.53 dS/m, respectively. VW samples compared [4] with approached one nutrient composition is nearly the same.

Hence, that approach VW can be used to hydroponic as a culture medium. NPK content of referred sample was 0.01-0.001%, 1.70% and 26 ppm respectively. NPK content of approached one was 0.012 - 0.005%, 1.85%, 25 ppm respectively. So, the main nutrient component of VW respectively is the same as other compared VW. Compared with the results obtained, the nitrogen content of the substitute VW samples was 0.01-0.001%, and the nitrogen content of the vermiwash was 0.012-0.005% [4]. The phosphorus content of the replacement VW was 1.70%. Further, the phosphorus value of the approach VW was 1.85% which had increased by a small amount. Also, the potassium value of the substitute VW was 26 ppm, and the potassium value of the approach VW was 25 ± 2 ppm.

VW is an organic nutrient solution, and Albert solution is not. The only downside of the VW is the low growth rate of plants due to the lack of necessary nutrient components since its nutrient content depends on what is available with the food waste. When comparing the nutritional data of the AS with the approach VW, it is clear that the nutrition content of the VW is less than the nutritional content of AS. Therefore, there are few differences compared with the commercially available nutrient solution with VW that approach VW. They compared the growth rate of commercially available nutrient solution (AS) with VW; AS growth rate was higher than VW growth rate, and VW is completely cost-free compared with AS.

Conclusions and Recommendations

This ensures that the nutrient solution has all the basic nutrients needs to grow the plant. Therefore, the crops can be grown in the proposed VW medium. However, VW can be used as a medium for hydroponic cultivation. Moreover, that would be a more innovative solution to use food waste to create VW and use that medium to grow organic food. This method can be used as a cheaper and more valuable answer to food wastage.

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FOCUS AREA
Health

THE EFFECT OF STORAGE TEMPERATURE AND TIME DURATION ON OSMOLALITY, PROTEIN LEVEL AND pH OF URINE

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Introduction

Urine is a biological waste material. It is typically a sterile liquid by-product of the body containing metabolic breakdown products from a wide range of foods, drinks, drugs, environmental contaminants, endogenous waste metabolites and sometimes bacterial by products. It is secreted by the kidneys through a process called urination and excreted through the urethra [1]. Urinalysis is the third major *in-vitro* diagnostic screening test. According to the CLSI GP16A3 guidelines, the recommended time for urine analysis is within 2h of collection [2]. Delay in analysis and storage conditions may affect the accuracy of the results [3, 4, 5]. However, delays in transportation and analysis of urine are common as sometimes samples are collected in peripheral centers and are analyzed in one central laboratory, or due to large number of samples receive to analyze with limited human resources available when it comes to countries like Sri Lanka. Thus, this research was designed to study the impact of time and temperature variations for selected parameters in urine analysis. The study attempts to analyze the impact of time and temperature on osmolality, Protein and pH of urine.

Materials and Methods

An experimental, cross-sectional study was performed using 50 urine samples from the patients attended Teaching Hospital Karapitiya. First aliquot the sample in to nine and then the tests were performed. pH was measured by pH meter (Hanna instruments HI8424 microcomputer) and urine osmolality was measured by the osmometer (OSMOMAT 030 GenolecCryoscopic) under different storage conditions and time intervals. Urine protein content was measured semi quantitatively, by using 10 parameter urine strips (UroColor™ 10Test strip for urinalysis).

Patients' request forms were used to collect data regarding patient information, sample collection and sample receiving times. A screening questionnaire was used to collect information on patients' dietary habits, medications and supplement of vitamins etc. Information on disease conditions and previous day

diet composition were collected to avoid possible errors. Patients those who have any diet or medication that might have impact the Urine report were excluded from the study. Data was analyzed SPSS software version 25. Used Descriptive statistics (Mean, median, standard deviation) and the repeated measures function in SPSS.

Urine samples with an adequate volume (more than 25 mL), samples collected following proper advices, sample received and analyzed within 2 hours since collection, samples collected, transported and stored at room temperature (between 25-37 °C) were included for the study.

Any urine sample presenting any abnormal color, any contaminated urine samples, urine samples from patients who are on high doses of certain vitamins (B and C) were excluded from the study.

Results and Discussion

pH

Among the studied sample no significant pH changes were observed compared to spot sample when samples were analyzed after 3 hours, 6 hours and 24hours kept at room temperature (25-37 °C) or at 2-8 °C (refrigeration) ($p>0.05$).

However, pH had been increased ($p<0.05$) in samples stored at room temperature after 48 hours where as it was not changed in the samples stored under refrigeration (2-8 °C) at 48 hours.

Osmolality

Osmolality was not changed significantly under both storage conditions. i.e., at 2-8 °C or at 25-37 °C up to 48 hours since collection ($p>0.05$).

Table1 illustrates the mean (SD) pH and the osmolality of the urine samples stored under two different temperature conditions with 3, 6, 24 and 48 hours time intervals.

Table 1. pH and osmolality changes of the urine samples stored at two different temperatures over the time

Sample	pH	Osmolality (mOsmol/kg)
	Mean (SD)	Mean (SD)
Spot sample	4.87 (0.47)	498.50 (221.91)
3 hours room stored	4.88 (0.47)	498.38 (222.08)
6 hours room stored	4.89 (0.48)	498.20 (221.38)
24 hours room stored	4.91 (0.54)	501.14 (221.84)
48 hours room stored	5.25(0.89)*	497.28 (223.22)
3 hours refrigerated	4.88 (0.47)	497.96 (221.87)
6 hours refrigerated	4.88 (0.47)	497.36 (222.89)
24 hours refrigerated	4.90 (0.49)	510.06 (228.45)
48 hours Refrigerated	4.92 (0.49)	506.10 (232.39)

*p<0.05 in urine sample stored at room temperature after 48 hours

Protein

Protein was examined semi quantitatively. Forty-two urine samples were negative for protein.

Protein levels of positive samples were not changed significantly, in the room temperature up to 24 hours and in refrigerated samples up to 48 hours (p>0.05). However, protein levels of samples stored 48 hours at room temperature was decreased significantly (p<0.05).

Conclusions and Recommendations

According to the studies sample, the accuracy of urine osmolality can be maintained up to 48 hours either if the samples are stored at room temperature or in the refrigerator (2-8 °C). The pH value appears to be inaccurate when it is measured after 24 hours stored at room temperature.

Urine protein concentration also appears inaccurate in urine samples stored at room temperature after 48 hours of collection. However, the accuracy of the protein concentration can be maintained up to 48 hours after the collection when the urine sample is stored in the refrigerator (2-8 °C).

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A CROSS-SECTIONAL STUDY ON MATERNAL FACTORS FOR STILLBIRTHS TAKING PLACE IN HOSPITALS IN KANDY, SRI LANKA

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Introduction

Stillbirth, a baby born with no signs of life at or after 28 weeks gestation, can be resulted from preventable maternal conditions such as non-communicable diseases, infections and pregnancy complications [1,2]. Low- and middle-income countries are accountable for 98% of the stillbirths around the world. Among those, south Asia and sub-Saharan Africa reports three fourth of the stillbirths [2]. Number of studies which describe the situation of stillbirths in Sri Lanka are very few. Stillbirths have been underreported although the government spends a large amount of money from the budget to improve and delivery free health care to the people. The stillbirth rate of Kandy district depicted a downward trend with fluctuations which also showed a rise in 2017 where the rate was greater than the average stillbirth rate of Sri Lanka [3].

Advanced maternal age, unplanned pregnancy, mother's ethnicity, low educational level of the mother, type of maternal occupation, low economic status, being unmarried or in an extramarital relationships, consanguinity, maternal medical conditions, substance abuse, abdominal trauma during pregnancy, increased maternal body mass index (BMI) and underweight mothers, maternal anemia, and intrapartum complications significantly contribute for stillbirths of which many of them are preventable [1,2].

Therefore, we aimed this study at determining the prevalence of maternal characteristics that contribute to stillbirths that occurred at Kandy district hospitals, Sri Lanka.

Materials and Methods

This descriptive cross-sectional study conducted in Kandy district, Central Province, Sri Lanka for 12 months from April 2017. The participants of this study were the mothers from who delivered stillborn baby/babies following a minimum of 22 weeks of gestation, at government hospitals of Kandy district. There are 23 Medical Officer of Health (MOH) areas, 73 primary health care institutions, 2 secondary care institutions, 3 tertiary care institutions and 9 special units/

campaigns in the Kandy district by 2009. Base, district, general, and teaching hospitals were included in the study.

The sample size was calculated according to the following formula.

$$n = Z^2_{1-\alpha/2} P (1-P) / d^2$$

The prevalence of three variables were considered and the population proportions were estimated with specified absolute precision of 0.05 and at 95% confidence level. The largest sample size was calculated as 246. Therefore, for this study 246 of mothers were considered as the total sample size. All consecutive cases of stillbirths were included in the study from the beginning of the study till the required sample size was invited.

Ethical clearance was obtained from the Ethical Review Committee, Faculty of Medicine, University of Peradeniya. Permission to collect data was obtained by the Director/Medical Superintendent of the hospitals, consultants and In-charge nurses. Informed consent was obtained from each mother who participated in the study prior to collect data.

A structured questionnaire was used for the data collection which was validated by two obstetricians and gynecologists. Maternal factors collected by interviewing the mother directly were age of the mother, ethnicity, religion, level of education, type of occupation, total monthly income, marital status, planning of pregnancy, and consanguinity. Through the previous diagnostic records and the bed head ticket of the mother, data regarding known existing medical conditions, antepartum medical complications and intrapartum complications, substance abuse, infections during pregnancy, and trauma during pregnancy were gathered. Records on BMI, intake of nutrient supplements, and hemoglobin (Hb) level were collected through antenatal record of the mother. All data was entered in the SPSS 19 program for analysis. Monovariate analysis was carried out to describe the study sample. Frequency (N), percent (%), mean, median, standard deviation (SD), minimum, maximum and 95% confidence intervals (CI) were calculated for continuous variables. Frequency and percent were determined for rest of the variables.

Results and Discussion

A total of 246 mothers participated in this study. Mean age of the mothers who experienced stillbirths were 29.3 years and majority of them were between 25 and 29 years (n=77, 31.3%) of age (Table 1). About 68% were Sinhalese and Buddhists. Most of the mothers educated up to O/L education only and were unemployed. Mean income was around LKR 40,000.00. There were six single mothers. About 68% mothers have planned pregnancies. Though, majority did not have consanguineous marriages, 19 (7.7%) mothers reported consanguinity.

Table 1. Descriptive statistics of socio-demographic factors of mothers

Character	N (%)	Mean	Median±SD	Minimum, Maximum	95% CI
Age (years)*	246 (100)	29.3	29±5.8	17, 45	28.6-30.0
<20	9 (3.7)				
20-24	45 (18.3)				
25-29	77 (31.3)				
30-34	62 (25.2)				
35-39	45 (18.3)				
40±	8 (3.3)				
Ethnicity	246 (100)				
Sinhala	168 (68.3) 47				
Tamil	(19.1)				
Muslim	31 (12.6)				
Religion	246 (100)				
Buddhist	168 (68.3)				
Hindu	41 (16.7)				
Islam	31 (12.6)				
Christians	6 (2.4)				
Years of education (years)*	^a243 (98.8)	11.8	11±2.3	3, 17	11.5-12.1
1-5	3 (1.2)				
6-9	23 (9.3)				
10-11	118 (48)				
12-13	65 (26.4)				
> 13	34 (13.8)				
Occupation	246 (100)				
Unemployed	192 (78)				
Professionals	17 (6.9)				
Technicians	2 (0.8)				
Clerical	16 (6.5)				
Sales workers	4 (1.6)				
Craft workers	6 (2.4)				
Elementary	9 (3.7)				
Total monthly income**(LKR)	^a236 (95.9)	41144.1	35000±	10000,	36884.0-
		0	33218.60	300000	45404.1
Marital status	246 (100)				
Married	240 (97.6)				
Single	6 (2.4)				
Planning of pregnancy	^a240 (97.6)				
Planned	166 (67.5)				
Unplanned	74 (30.1)				
Consanguinity	246 (100)				
Yes	19 (7.7)				
No	227 (92.3)				

*. normally distributed

**..non normally distributed

^aN<Total number did not cover the whole sample due to unavailability of data.

Among existing medical conditions, the most reported was anemia (n=31, 12.6%) while bronchial asthma (BA) (n=19, 7.7%) was the second commonest. Pregnancy induced hypertension (PIH) (n=44, 17.9%) was the commonest pregnancy induced disorder reported followed by gestational diabetes mellitus (n=28, 11.4%). As a whole, hypertensive disorders were diagnosed in 55 (22.4%) mothers which represented more than one fifth of the sample. Diabetic disorders were diagnosed among 40 (16.3%) mothers. Betel chewing was reported by 2% (n=5) of women. No mothers had trauma during pregnancy were reported.

A significant number of mothers were deviated from normal range of BMI (Table 2). Mean BMI was 23.4. Seven (2.8%) mothers were severe underweight and 21 (8.5%) mothers were obese.

Table 2. Body mass index of mothers

BMI Category	N (%)	Mean	Median± SD	Minimum, Maximum	95% CI
BMI (kg/m ²)*	^a 220(89.4)	23.4	23.3±4.7	12, 47.6	22.8- 24.1
<16 (severe underweight)	7 (2.8)				
16-16.9 (moderate underweight)	7 (2.8)				
17-18.49 (mild underweight)	13 (5.3)				
18.5-24.9 (normal range)	115 (46.7)				
25-25.9 (overweight)	57 (23.2)				
≥30 (obese)	21 (8.5)				

^aN< Total number of cases due to unavailability of data

*Variables in this table were normally distributed

No any mothers were diagnosed as positive for syphilis (Table 3). Two mothers were severe anemic. More than three fourth were non-anemic. Mean Hb was 11.8 g/dL (Median=11.9, SD=1.4, Minimum=3.7, Maximum=15.2, CI=11.7-12.0). The majority of mothers took iron and folate (n=242, 98.4%), calcium (n=234, 95.1%) and vitamin C (n=234, 95.1%) during antenatal period.

Table 3. Antenatal findings of the mother

Antenatal clinic component	N (%)	Mean	Median±SD	Minimum, Maximum	95% CI
Iron / Folate	^a242(98.4)				
Yes	235 (95.5)				
No	7 (2.9)				
Calcium	^a241(98)				
Yes	234 (95.1)				
No	7 (2.9)				
Vitamin C	^a241(98)				
Yes	234 (95.1)				
No	7 (2.9)				
Syphilis screening	^a241(98)				
Negative	232 (94.3)				
Positive	-				
Not done	9 (3.7)				
Hb level at first clinic visit (g/dl)*	^a234(95.1)	11.8	11.9±1.4	3.7, 15.2	11.7-12.0
<7 (severe anemia)	2 (0.8)				
7-9.9 (moderate anemia)	12 (4.9)				
10-10.9 (mild anemia)					
≥11 (non-anemic)	29 (11.8)				
	191(77.6)				

*Variables in this table were normally distributed

**Variables in this table were non normally distributed

^aN< Total number of cases due to unavailability of data

Considering age of mothers who participated in the current study, the most frequent age group was 25-29 years (31.3%). Comparing this study results with worldwide findings, the developed and upper-middle income countries records were similar to present study findings [1]. The significance of this finding is that expecting the majority to represent either advanced maternal age or adolescents. However, our study convinced that fact cannot be generalized. It is an important finding which illustrates the quality of health care delivered to the mothers in the country.

Since the highest percentage of the Sri Lankan population comprises Sinhalese, as our study depicted the same, there is no significant difference between country's current statuses.

Among the participants, 1.2% of mothers had attended up to Grade 5, 9.3% up to grade 9, which is lower than women in the general population in Sri Lanka [4]. However, percentage of mothers who studied up to grade 10 and 11, grade 12 and 13, and tertiary education was higher than of the general population in our study. This brings a challenge to the professionals of our health care system to

figure out the actual problem existing among these literate mothers to face stillbirths, whether it is related to the knowledge of healthy behaviours during antenatal period or any other inevitable reason has been occurred. Further, 78% of mothers were unemployed followed by professionals (6.9%) with a vast gap between those two. In our study, mean income was LKR. 41144.10, and median was LKR. 35,000.00 was higher than Sri Lankan average. This may have been due to high income level of few families included in the study. Unemployment is one of the triggering factors for poverty. Lack of financial assistance may cause poor compliance in following antenatal clinics, and facing issues in both procuring essential foods and supplements and fulfilling other health seeking behaviours for pregnant women whenever needed.

As the number of mothers who had consanguinity marriages was very less, it is obvious that the knowledge regarding health promotion has been improved with time.

Occurrence of non-communicable disease during gestational period was noticeable among the participated mothers of this study. Diabetes mellitus and hypertensive disorders and complications of hypertensive disorders were among diagnosed disorders. The number of mothers suffered from bronchial asthma in the study was high. As these disorders directly associated with pregnancy complications and have adverse effects on the fetus, proper follow up of the mothers is emphasized.

Only five mothers were chewing betel during pregnancy but without adding tobacco. It is already known fact that betel chewing causes oral cancers. However, further research is needed to identify any relations of it with fetal complications.

An obvious number of mothers were underweight, overweight and obese. Anemic conditions were also not uncommon among these mothers. These results imply both nutritional deficiencies and excessive fat levels prevailing among the women. Dietary habits must be modified specifically for these mothers. Health care personnel have the responsibility to educate these mothers regarding weight gain and weight loss according to their BMI and obtaining healthy meals as they can afford. However, just the instructions will not make it success unless service providers monitor the women's adherence to them.

Conclusions and Recommendations

Being 25-29 years age, education up to or below grade 11, being unemployed, suffering from chronic hypertension or pregnancy induced hypertension, chronic diabetes mellitus or gestational diabetes, underweight, overweight, obesity, nulliparous, suffering from anemia, are most common maternal factors found in stillbirth population. Pre-pregnancy counseling should be started from the adolescence and continued to all women in reproductive age group including

initiation of folic acid supplements to be considered from the puberty of the female. Awareness on life style modifications and nutrition & diet review must be provided and encouraging mothers to take “Poshana Malla” from each clinic are emphasized.

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***In-silico* MODELING OF MgO NANOCARRIER PHARMACOKINETICS AND TUMOR DELIVERY - INFLUENCE OF SURFACE CHARGE**

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Introduction

A cancer medication chemotherapy is toxic to the healthy organs, and a tiny amount of dosage can find in the tumor site, and nanocarriers can emit these drawbacks. Amid the numerous nanocarriers, MgO nanoparticles (NPs) manifests biocompatibility and can be dressed from economical precursors. The experimental trial and error methods present less perception of the nano-bio interplay thus, consume more time and financiers. Therefore, The element *in vivo* interactions of adsorption, distribution, metabolism, and excreta (ADME) present in the computational based method, physiologically based pharmacokinetic (PBPK) modeling approach, simulates concentration variation with time in any organ described as compartments. The nano vectors limited clinical application inducing to biological barriers, and the most significant barrier is passing through the endothelial and epithelial surfaces and interiorized to the cell endocytosis [1]. In the *in vitro* and *in vivo* studies, surface-charged NPs expose a higher affinity to merging to the cell lamina. In this paper, renew the Dogra et al. [2] model with the traits of neutral and positively charged MgO NPs and critique the influence for pharmacokinetics and drug delivery system (DDS). Sensitivity analysis proffers the influence of the perturbations of the model parameters in the model output in an analysis of the complex biochemical reaction systems.

Materials and Methods

Governing equations in body compartments

The sedimentation velocity (v') of the charged nanoparticles (NPs) in the blood vessel wall was derived using Stokes law Peclet number, where, g is gravitational acceleration constant, r is NP radius, ρ_{NP} and ρ_P is density of NPs and plasma, respectively, μ_i is dynamic viscosity of blood in i^{th} organ, E is the electric field strength of the blood vessel, q is the NP charge, D is effective diffusivity of NPs, an l, u and R are the length, blood flow rate and radius of the capillary, respectively.

$$v'_{corrected} = \left(\frac{2}{9} \times \frac{gr^2(\rho_{NP}-\rho_P)}{\mu_i} + \frac{Eq}{6\pi\mu_i r} \right) \times \frac{Dl}{uR^2} \quad (1)$$

The characteristic parameter of the NPs deposition k_{on} can be obtained from the extended sedimentation velocity component which has the linear relation with NP charge:

$$k_{on} = \frac{v'_{corrected}}{R} \quad (2)$$

The derived k_{on} applying into the equation describes for the tumor and the healthy organs in mentioned in prior literature [3] and solved using stiff ode solver.

Sensitivity analysis

NP, tumor, and individual-related 12 parameters (domain x) elected from preceding literature [2] and randomly preferred nominal values were appraised under the sensitivity analysis (Table 1). The area under the curve ($AUC_{0 \rightarrow \infty}$) of the tumor compartment was analyzed by comparing the NPs whether or not with exterior charge, independently.

Table 1. Wielded parameters

Parameter	Symbol	Nominal value
MgO NP radius (nm)	r_{NP}	20 [3]
MgO density (g /cm ³)	ρ_{NP}	3.587 [3]
MgO NP charge (C /m ³)	q	3 [4]

Local sensitivity analysis (LSA)

The local sensitivity analysis (LSA) was performed to obtain the influence of the inputs linearly or additively with $\pm 99\%$ perbutation from the reference value. The restriction of the domain x at a minimum and a maximum value, triangular distribution was practiced for 1000 randomly chose levels within the given range. The sensitivity coefficient (SC) concludes the perbutation of the per parameter influence in MgO accumulation in the tumor chamber.

$$SC = \frac{AUC'_{0 \rightarrow \infty} - AUC_{0 \rightarrow \infty} / AUC_{0 \rightarrow \infty}}{Par' - Par / Par} \quad (3)$$

where $AUC'_{0 \rightarrow \infty}$ and $AUC_{0 \rightarrow \infty}$ is the area under the curve of the perbutation value(Par') and the reference value (Par').

Global sensitivity analysis

Normally distributed random numbers were employed for the 12 parameters in 10000 arbitrary sample runs in Monte Carlo simulation. The mean and standard deviation (std) were practiced to observe the mean MgO NP concentration in the extravascular compartment and whence considerable differences each run from the mean value whilst the parameter interaction was in consideration.

Results and Discussion

Influence of charge on tumor accumulation

The prior literature [2] NPs (Nominal NPs) with 50 nm radius and the 2 g/cm^3 density, concentration in the plasma, tumor vascular, extravascular and bounded show in figure 1 with neutral and the charged surfaces concluding charge hasn't influence in plasma. When combining the surface charge into these nominal NPs (figure 1 (d)), the vascular chamber has zero concentration, and in the meantime in the extravascular chamber in $\sim 1 \text{ min}$. NP consistency increases to $0 \text{ } \mu\text{g/ml}$ to $\sim 60 \text{ } \mu\text{g/ml}$ and remains constant.

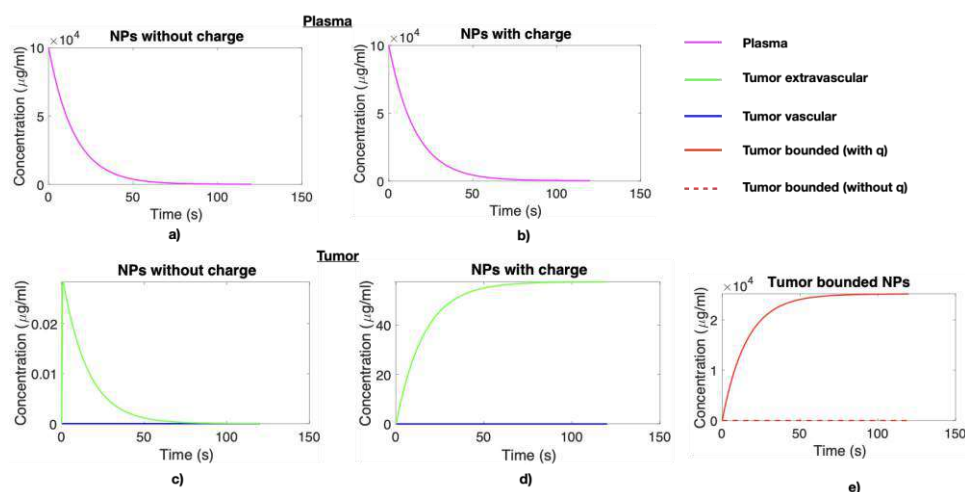


Figure 1. Nominal NPs concentration in the plasma, tumor vascular, extravascular and bounded without and with the surface charge

The gain in charged NPs concentration may occur owing to the strong affection between the negatively charged luminal surface of the blood vessel plus the positively charged large NPs surface and disengage of the considerable size NPs. It's heavily justified by figure 1 (e), and it shows the significant increase in the energized bounded NPs concentration. The attached NPs to the blood cells may detach due to the large NP dimension, and they may trap in the extravascular matrix, increasing their particle concentration.

Figure 2 illustrates the MgO NPs delivery in plasma, tumor extravascular, vascular and bounded matrix in the presence and absence of the exterior charge. Figures 2 (a), (b), and (c) mirrored the corresponding profile in nominal NPs thus, the zero concentrations achieve at different times. The charged MgO shows a similar configuration as the neutral MgO profile in extravascular, obtaining high concentration with a lower decreasing rate and therefore, attaining zero amount in $\sim 1 \text{ min}$.

The bounded MgO NP concentration reveals the figure 2 (e) with the existence and non-existence of the charge. The nominal NPs have higher affection than the MgO presence of the charge (Figure 1 (e) and 2 (e) solid lines) and bounded MgO NPs withdraw from the luminal layer while nominals survive stationary. The 10 nm particles easily move toward and trap in flowing RBCs compared to the 50 nm particles and which may cause the detachment of bounded MgO NPs from the tumor blood vessel wall. This may also influence by the negatively charged RBCs, thus which charge potential is less than the endothelial cell layer [5].

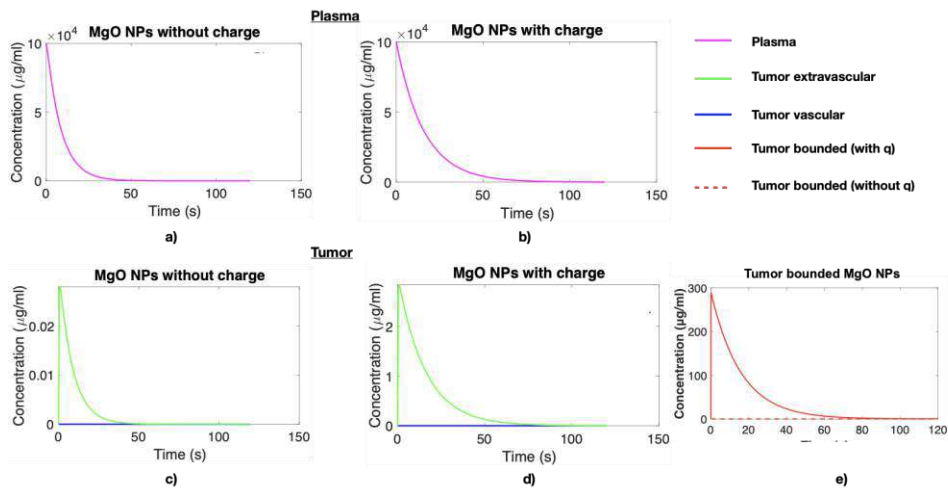


Figure 2. MgO NPs concentration in the plasma, tumor vascular and extravascular without (a) and with (b) the surface charge.

Local sensitivity analysis

The results of sensitivity coefficients shown in figure 3, and lower value 1 and higher value 12 rankings are used. It concluded the NP radius and degradation rate have the highest effect on NP accumulation in the extravascular matrix, meanwhile, blood viscosity, the area fraction of spleen, and NP exterior charge have a moderate impact.

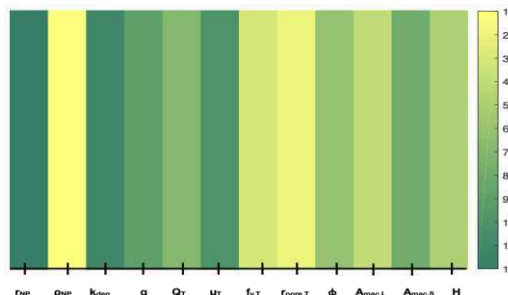


Figure 3. Parameter ranking in line with sensitivity coefficient (SC)

Global sensitivity analysis

The neutral ones mean plus std are 0.0010 g/cm^3 and 0.0024 , respectively. Meanwhile, the charged ones have $6.014 \times 10^{-4} \text{ g/cm}^3$ mean and std as 0.0022 . It presumes the presence of charge in MgO NPs limits the tumor interstitium delivery while the parameters have their interplays, thus, each count departure from the mean amount is comparatively low.

Conclusions and Recommendations

The 12 parameters that influence the charged NPs accumulation in the tumor compartment were analyzed and NP size, degradation rate, blood thickness conclude being the numerous decisive parameters in the DDS in tumor interstitium. Coating the surface charge in NPs improves the delivery to the extravascular thus, non-sufficient. The researchers experimented with substantial adequate delivery in the charge-reversing NPs utilization owing to the various physiological conditions' *in vivo* studies. For the future viewpoints, accounting these biological provisions in mathematical and computational modeling employing the tuning the NP charge.

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DESCRIPTIVE CROSS SECTIONAL STUDY OF RISK FACTORS AND THEIR ASSOCIATIONS AMONG ADULT ASTHMATIC PATIENTS ATTENDING SELECTED CHEST CLINICS IN COLOMBO, SRI LANKA

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Introduction

Asthma is a serious global health problem affecting all age group, with increasing prevalence in many developing countries [1]. It is a heterogenous and common chronic respiratory disease, characterized by chronic airway inflammation [2]. Globally, asthma is ranked sixteenth among the leading causes of years lived with disability and twenty-sixth among the leading cause of burden of disease [3]. In Sri Lanka, 2.5 number of deaths per 100 000 population is due to asthma and a higher prevalence of asthma can be observed among adults rather than children [3]. Uncontrolled asthma is identified as common and remains a frequent cause of hospital admission [4]. The aim of this study was to identify the risk factors and their associations among adult asthmatic patients those who attend two chest clinics in Colombo, Sri Lanka.

Materials and Methods

A descriptive cross-sectional study was conducted among 180 asthmatic patients who were attending two selected chest clinics in Colombo, Sri Lanka. Patients with acute episodes of asthma, patients with acute episodes with secondary infection (presently on antibiotics) and patients with other comorbidities such as diabetes and hypertension are excluded.

Study sample calculation

Consecutive sampling method was used as the sampling method. Sample size was calculated using the following formula.

$$n = \frac{z^2 p(1 - p)}{d^2}$$

n = sample size

z = 1.96; standard normal deviation for the normal chosen confidence level. (Chosen confidence level is 95%)

p = expected proportion of the subjects with the characteristics were studied d = 0.05; margin error

According to the previous studies and annual health statistics, overall prevalence of self-reported asthma was 11.8% in Sri Lanka [1]. Therefore, the sample size was calculated as follows,

$$n = \frac{1.96^2 \times 0.118 \times 0.88}{0.05^2}$$

$$n = 159.56$$

Therefore, sample size was 160 subjects. Leaving for 10 % of dropouts the total sample size 176 subjects.

Data collection instrument

An interviewer administered questionnaire used to determine base line data. Anthropometric Measurements (Weight, Height), Measurements of respiratory function (PEFR level, spirometric (FEV1, FVC and FEV1/FVC), Measurement of air way inflammation - FeNO level were obtained. Asthma control test used to detect how well the patients' asthma has been controlled over the last four weeks. There are 5 questions and give score out of 25. Quality and patterns of sleep were studied using the validated Pittsburgh Sleep Quality Index questionnaire. The physical activity levels were determined using the validated International Physical Activity Questionnaire.

Ethical clearance

Ethical clearance was obtained from the Ethics review committee of Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda and Colombo South Teaching Hospital, Kalubowila and permission to carry out the study at Base hospital, Homagama was obtained from the director of the hospital.

Data analysis

Data was analyzed using SPSS software version 25. Descriptive statistics such as mean, median and Standard Deviations (SD) was used to summarize the result. Categorical variables were depicted as numbers, percentages and continues variables we represented as means and standard deviations. Frequency of socio demographic Characteristics, types of asthma medication, type of inhalers they used were analyzed by using descriptive statistics. Asthma control also scored as out of 25. Overall asthma control rate as ≤15 uncontrolled, 16- 19 partially controlled, 20-24 controlled and 25 completely controlled. The PSQI includes a scoring key for calculating a patient's seven sub scores, each of which can range from 0 to 3. The final sub scores can range from 0 to 21. A global score of 5 or more indicates Deficient sleep and score of less than 5 indicates healthy sleep. International physical activity questionnaire was analyzed according to the guidelines for data processing and analysis of the international physical activity

questionnaire. The continuous score allows the estimation of the weekly energy expenditure expressed in weekly MET minutes or MET-min/week. This is obtained by multiplying value of energy expenditure for the given physical activity. The categorical score classifies individual in to three categories. Low physical activity (less than 600 MET-minutes/week), Moderate physical activity (600- 3000 MET-minutes/week) High physical activity (more than 3000 MET-minutes/week). Multivariate logistic regression was employed to examine the association between variables ($p < 0.05$).

Results and Discussion

The majority (60%) were females. The prevalence of asthma in this study similarly to the study reported in Sri Lanka [1]. And also, the prevalence of asthma in Sri Lankan adults is high in comparison with global data [1]. The mean age of the patients was 41.8 years (± 15.54). In this study group only 34.4% of patients had family history of asthma. This may indicate that other factors other than genetic predisposition may contribute to asthma in this population [3]. There were 10% smokers. As per data from other countries, asthma patients in Sri Lanka too are substance abusers a majority of 10% were smokers despite their asthma [1]. Most asthma patients were users of inhalers, 56.7% of the population of the study were treated with inhalers 70% and used short acting beta 2 agonist as their inhaled drug. The mean BMI was $25.7 \pm 5.3 \text{ kgm}^{-2}$. Mean values of respiratory functions are PEF_R = $192.5 \pm 78.5 \text{ (L/min)}$, FEV₁ = $1.42 \pm 0.51 \text{ (L)}$, FVC = $2.07 \pm 0.56 \text{ (L)}$, FEV₁/FVC (%) = 0.67 ± 0.14 , FeNO = $18.47 \pm 16.01 \text{ ppb}$. According to the ACT scores, mean ACT score was 17.31 ± 3.32 . Only 33.33% of patients had controlled asthma (Figure 1). More than 66% of patients had uncontrolled and partially controlled asthma. Perhaps this may be due to poor involvement of patients in their disease, poor drug adherence, exposure to asthma triggers and deficient sleep. Age ($p = 0.04$), gender ($p = 0.01$), education level ($p = 0.03$), occupation ($p = 0.02$), family history of asthma ($p = 0.05$), smoking ($p = 0.00$), follow up visits ($p = 0.04$), sleep quality ($p = 0.00$), level of physical activity ($p = 0.02$), decreased FEV₁ ($p = 0.04$) and use of SABA (short acting beta2 agonist) alone as anti-asthmatic medication ($p = 0.00$) were significantly associated with uncontrolled asthma. In contrast to this study a study done in 2016 showed that there was no association between asthma control and age, gender, educational level and duration of asthma [2]. Sleep quality was a significant predictor of asthma control. Most Sri Lankan patients do not engage in regular physical activity and in most cases cultural beliefs and family members inhibit a patient from engaging in regular physical activity. But according to this study sleep quality and physical activity were associated with level of asthma control.

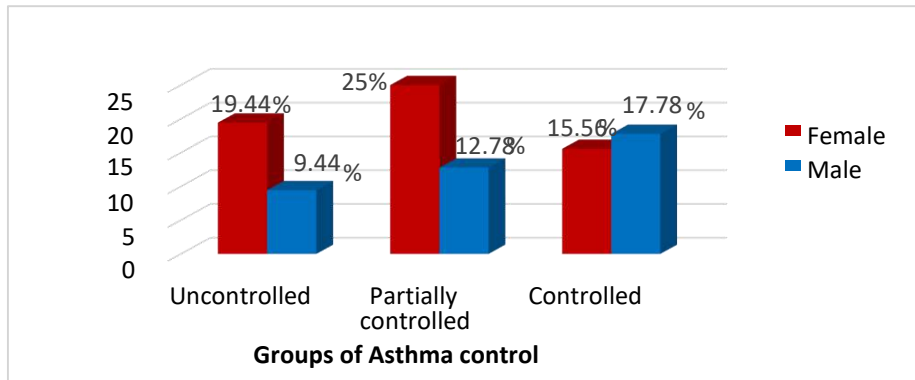


Figure 1. Asthma control according to gender distribution

Conclusions and Recommendations

The Majority of the asthmatic patients didn't follow recommendations of the international guidelines for asthma management [4]. In this study, majority of the patients had uncontrolled and partially controlled asthma and this was significantly associated with age, gender, education level, occupation, family history, smoking, follow up visits, sleep quality, level of physical activity, decreased FEV₁, asthma severity and use of SABA alone as anti-asthmatic medication. So, these factors associated with uncontrolled asthma may be considered as targets for future intervention strategies in Sri Lanka. Guidelines should recommend asking about sleep quality and encourage physicians to take a global sleep history from asthma patients. Patients should be motivated by health care personnel, during their clinic visits, to engage in regular physical activity.

Acknowledgment

The authors wish to thank patients and staff of the two hospitals and technician for the technical support.

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A LABORATORY BASED PREVALENCE STUDY OF JANUS KINASE 2 (JAK 2) V617F MUTATION

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Introduction

Janus kinase 2 (JAK2) gene codes for tyrosine kinase enzyme which initiates the cell proliferation and controls the blood cell production from haematopoietic stem cells. This is the main protein that is involved in the signalling pathway called JAK/signal transducer and activator of transcription (JAK/STAT) which transmits the signals from the cell membrane to the nucleus. JAK2 gene mutation occurs at chromosome 9p24 at the 1849 position by replacing G with T as a point mutation which causes the replacement of valine to phenylalanine in the 617 amino acid. [1, 2].

Mutation of the JAK2 gene mainly causes myeloproliferative neoplasms (MPN) disorders including polycythemia vera, essential thrombocythemia, primary myelofibrosis and indirectly enhances the possibility to get many other haematological malignancies. The JAK 2 mutation can be seen in 95% of patients who have polycythemia vera and 60% of patients with essential thrombocythemia and primary myelofibrosis [3]. Therefore, this mutation is considered the main genetic biomarker in haematological malignancies [1, 2].

The detection of the JAK2 mutation is important for the diagnosis of the above haematological malignancies. The quantification of the mutation is used to provide a better understanding of the diseases and enhances the ability to evaluate the drug therapies.

Many methods have been developed to detect this mutation namely, Restriction Fragment Length Polymorphisms (RFLP), pyrosequencing, real-time Polymerase Chain Reaction (PCR) and many other methods [1, 2].

This study was aimed to determine the prevalence of JAK 2 mutation in clinical specimens received to the Molecular Diagnostics laboratory of Lanka Hospitals Diagnostics (PVT) Ltd.

Materials and Methods

A total of 1196 patient samples that were received for the detection of JAK 2 mutation from January 2015 to January 2021 at the Molecular Diagnostics laboratory of Lanka Hospitals Diagnostics (PVT) Ltd were included in the study.

Ethical approval for this study was obtained from Ethics Review Committee, Lanka Hospitals Diagnostics (PVT) Ltd (Ref. No:90884). All the patients who were prescribed by haematologist to test JAK 2 mutation is the inclusion criteria for this study. All the other samples were excluded. The sample consisted of 948 males (79.26 %) and 248 females (20.74 %). The specimens received included both blood and bone marrow samples, with blood being the great majority. The data submitted with the samples were obtained from Laboratory Information Systems (LIS).

Human genomic DNA was extracted by the blood and bone marrow specimens using QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. The real-time PCR was followed by SYBR Green based method which uses the nonspecific dye (QIAGEN-SYBR Green, Germany) binding to any double-strand DNA and produces fluorescence and the specific primers (Integrated DNA Technologies, Inc., USA) to detect the JAK 2 mutation in the specimen. PCR inhibition was confirmed by using endogenous gene (glyceraldehyde-3-phosphate dehydrogenase -GAPDH) as an internal control and the assay specificity was increased using the melting curve analysis where T_m should be around 79–80 °C. The cut-off was set at Threshold Cycle (Ct) 40 which was the last cycle completely devoid of background noise.

All data was recorded on forms designed for each purpose. Data was processed in Microsoft Excel spread sheets (Microsoft office, 2010). Chi-square test (Statistical Analysis System- SAS) was used for comparison of categorical data. Two variables were analyzed at a 95% confidence interval and a p value <0.05 was considered as significant.

Results and Discussion

Out of 1196 samples, 157 (13.12%) were detected as positive for JAK 2 mutation and 1039 (86.88%) were wild type for the JAK 2 (Figure 1). Sixty-three (63) out of 248 females (25.4%) were positive for the mutation while only 94 out of 948 (9.91%) were positive among males. There was a statistically significant difference between male and female for JAK 2 positivity (mutant) ($\chi^2 = 41.3471$ and $p=0.00$ at a 95% confidence interval).

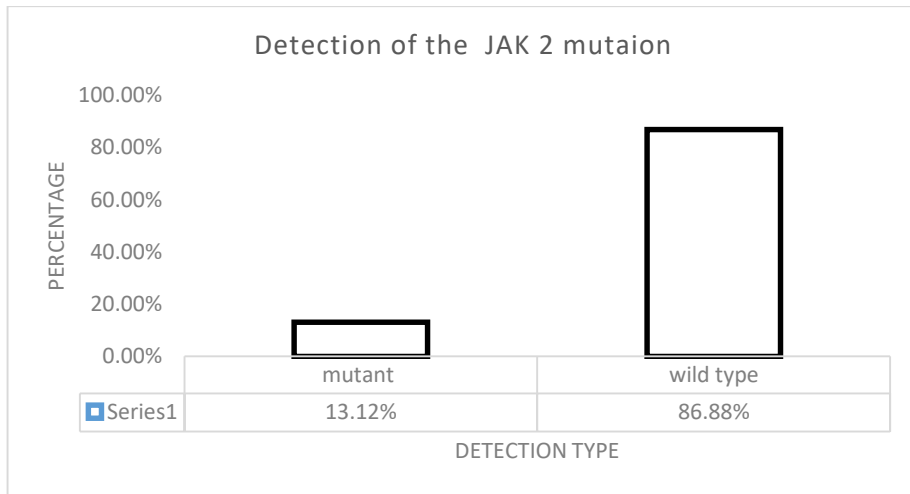


Figure 1. Detection of JAK2 mutation

Table 1. Age distribution of JAK 2 mutation among males and females

Age groups (years)	Female patients with the mutation N *(%)	Male patients with the mutation N *(%)
<=30	3 (4.77%)	9 (9.57%)
31 – 40	5 (7.94%)	8 (8.51%)
41 – 50	4 (6.35%)	15 (15.96%)
51 – 60	9 (14.28%)	25 (26.60%)
>=61	42 (66.66%)	37 (39.36%)

*N: Number

The table shows the number of patients who have been detected for the mutation and their percentage according to the gender differences. Out of the 63 mutant females, 42 were older than the age 61 years. In the male cohort, 37 out of 94 were mutant and were elder than age 61. The least mutant group of females was under the age 30 years. It can be seen that the number of mutants detected has been increased with the age.

Table 1 shows the variation of the mutation in the female and male with the age. The highest number of JAK 2 mutations can be seen in the female, age group above 61 and the male groups above 51. The least percentage of the mutation can be seen in the female age group under 31 and the male group between 32 - 40.

The prevalence of the JAK 2 mutation has been found to be 13.12% in our study population which has a lower prevalence than the research conducted with an Asian population with 34% prevalence since they have researched by using patients who have essential thrombocythemia. Our study cohort was patients who were without proper clinical history and which makes the reason for the difference between the prevalence [4,5].

In our study cohort, we have found that the elderly group which is above 60 has a higher percentage of mutant allele than that of the other age groups (Table 1), which is 39.36% in the male and 66.66% in the female. The prevalence of the Janus kinase mutation has been increased with the aging of the male and female populations. This has been proved by a few similar articles which were conducted to detect the JAK 2 allele burden and JAK 2 mutation detection of Asians. The results are slightly different due to the ethnic diversity and size of the study cohort. Most of the research had been carried out using fewer participants [4, 5].

Conclusions and Recommendations

The prevalence of the JAK 2 mutation among this study population is 13.12%. The male population carries a less mutant percentage (9.91%) for the JAK 2 than that of the female (25.04%). Research with a large sample size with the patients with myeloproliferative neoplasms and healthy individuals will be warranted for further evaluation of the correlation of the JAK 2 and the MPN.

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APPLICATION OF REAL TIME PCR FOR DETECTION OF BK VIRUS IN CLINICAL SPECIMENS

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Introduction

The BK virus (BKV) is a DNA virus common Polyomavirus of the *Papovaviridae* family. The primary infection of this virus occurs in childhood and its specific antibodies are detectable in 80-100% of individuals [1]. Although the majority of primary infection is asymptomatic or minimally symptomatic, asymptomatic reactivation with viruria occurs in immunocompetent individuals. Especially, BKV can cause nephropathy (BKVN) in renal transplant patients and leads to graft failure in 15% to 80% of patients. Moreover, BKV reactivation in bone marrow transplant patients may cause to hemorrhagic cystitis and patients with AIDS, chronic lymphocytic leukemia, and congenital immunodeficiency also severely effect by the BK virus reactivation [2].

Although, BKV infects a high proportion of the Sri Lankan population, scientifically sound data and scientific publications are also lacking in Sri Lanka. For a proper management of BKVN patients, specific antiviral therapy and definitive treatment for BKVN is needed at the early phase of the infection. Immunohistochemical test is considered as the method of definitive diagnosis for BKV infection, but the real time Polymerase Chain Reaction (PCR) has been widely used in recent years because it is a non-invasive way to identify patients at risk for BK nephropathy and to monitor response to therapy [3].

For the detection of BK virus, 2 types of samples can be collected: plasma from EDTA blood/bone marrow and/or urine sample. Urine samples are more prominent for the detection of BK virus in decreasing renal function or subsequent renal failure patients. The prognosis of BKVN is widely detected in urine samples as it helps on early detection of BKV infection, which also helps in early treatment for the patients [3].

This study was aimed to detect BK virus in clinical samples by real-time PCR for the determination of prevalence of BKV infection in clinically suspected patients in Sri Lanka.

Materials and Methods

Sampling

This study was conducted in the Molecular Diagnostic Laboratory of the Lanka Hospitals Diagnostics (PVT) Ltd from February 2015 to October 2021. Plasma (1168) and/or urine (497) samples of 1671 individuals clinically suspected of BKV infection were submitted to the laboratory. All necessary information was gained through the prescription form of the reference physician. Ethical approval was obtained from Ethics Review Committee, Lanka Hospitals Diagnostics (PVT) Ltd (Ref. No:90884).

PCR confirmation of BKV infection

DNA from plasma /urine was extracted using QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. real-time PCR using BKV - F primer (5'-GAAGCAACAGCAGATTCTCAACA-3') and BKV-R primer (5'-AGCAGGCAAGGGTTCTATTACTAAAT-3' [2,4] and the SYBR Green-I technology. Real Time PCR was performed using Primers on Rotor - Gene Q real time thermal cycler (Qiagen, Germany). Reaction conditions were optimized using reference samples obtained from the Super Religare Laboratories (SRL) Diagnostics, Mumbai, India. The cut off was set at threshold cycle (Ct) 32 which was the last cycle completely devoid of background noise. The first negative derivative of the initial melting curve (-dF/dT) was plotted against temperature for improved determination of the melting temperature (T_m)

Results and Discussion

This study consists of 358 (21.42%) female patients and 1313 (78.57%) male patients. The results showed that 306 of 1671 individuals (18.31%) were positive for BK viral infection. Among the positive individuals, 202 (66%) were males and 104 (34%) were females (Figure 1). Among the different age groups, the highest incidence of BKV infection of 26.50% was observed in age groups of 50-59 years (81), 60-69 years (79; 25.81%), 40-49 years (39; 12.74%), 71 years and above (46; 15.03%), 30-39 years (32; 10.45%), 20-29 years (26; 8.49%), and 10-19 years (03; 0.98%) (Figure 2).

Gender distribution of BKV positive cases

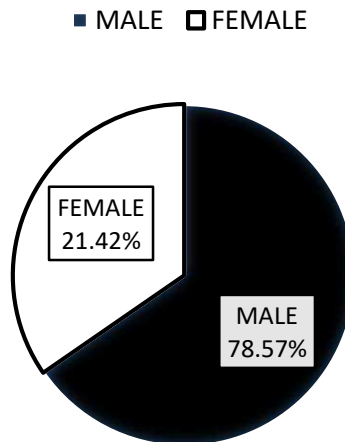


Figure 1. Percentage of BKV detected cases among female and male patients

Out of 1671, 497 samples were urine samples. One hundred fifty (150; 30%) of 497 urine samples were positive for BKV infection. On the other hand, only 156 (13%) out of 1168 plasma samples were positive for BKV. According to our findings that the percentage of BKV detected cases in urine is higher compared to the detected cases in plasma.

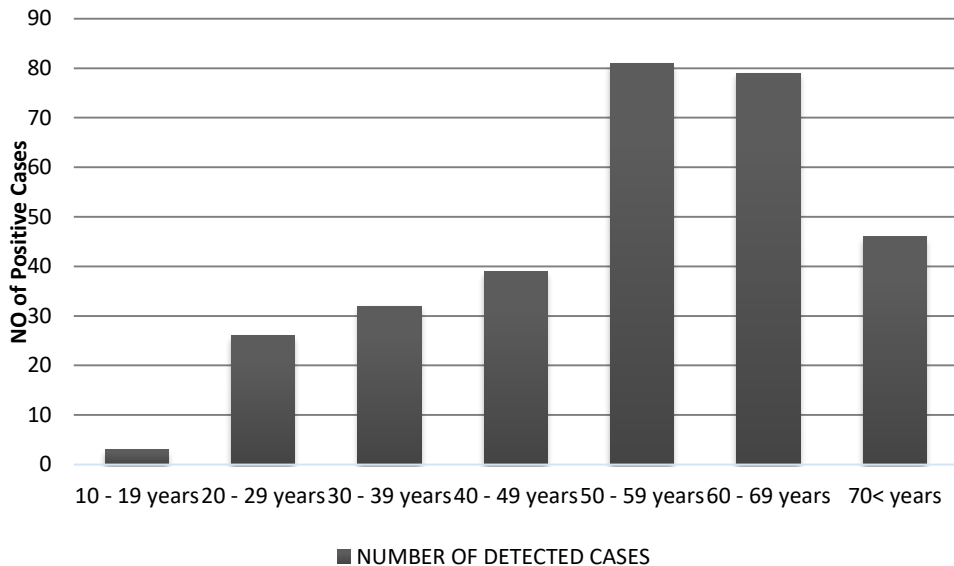


Figure 2. Age distribution among BKV positive cases

However, according to the recent studies of Satoru Kudose and Jianli Dong [3] and Chung et al., [5] the testing of urine and plasma samples of each patient is important because plasma and urine shows different lower limit of detection values. Although, this study is limited to by its design, PCR from one of this specimen helps in early detection for the prevention of BKV nephropathy. The initial detection of BKV in plasma could indicate that the patient is already in risk of BKV nephropathy [1].

Conclusions and Recommendations

The PCR is a non-invasive method which is important to screen the evidence of BKV infection in order to prevent irreversible graft damage of BKVN for proper management of BKVN patients. We believe that PCR will be a valuable tool for early definitive laboratory diagnosis of BKV with 100% specificity.

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APOPTOSIS-RELATED GENE EXPRESSION IN HUMAN RHABDOMYOSARCOMA (RMS) CELLS TREATED WITH A SRI LANKAN RED SEAWEED *Gracillaria edulis* (Gmelin) Silva

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Introduction

Cancer is one of the most serious health problems worldwide and is characterized by uncontrolled cellular growth with metastasis lesions. It is the second leading cause of death worldwide. Among the different types of cancer, rhabdomyosarcoma is one of the most abundant soft tissue sarcoma found in children below 10 years [1]. As higher incidences of cancers are reported, it has been proved that the available therapeutic interventions have several side effects. Therefore, it is essential to search for an effective drug that may benefit patients suffering from different types of cancers. Marine seaweeds are a rich source of bioactive metabolites that exhibited potent biological activities [2]. As polyphenols in marine algae are a rich source of antioxidants, they have the ability to combat most of the degenerative diseases. *Gracillaria* is a red alga that belongs to the family Gracilariaceae. Therefore, in lieu of herbal drug development, natural antioxidant-rich marine seaweeds can be utilized for the development of potential anti-cancer therapy. Therefore, the present study was aimed to analyze the apoptotic-related gene expression of human rhabdomyosarcoma (RMS) cells treated with hexane fraction of *G. edulis*.

Materials and Methods

Collection of alga sample (Gracillaria edulis)

The permission to collect algae samples was obtained from the Department of Wildlife Conservation (permit number- WL/3/280/17). *Gracillaria edulis* was collected from the Kalpitiya. The collected samples were cleaned and washed with fresh water to remove salt, sand, attached epiphytes, and organic matter. The samples were air-dried and ground into a fine powder and stored at -20°C until further use.

Preparation of G. edulis extracts and fractions

Homogenized *G. edulis* powder (10.0 g) was extracted three times using 70% methanol, subjected to sonication at 25 °C for three 90 minutes periods. The polyphenols were separated by precipitating crude polysaccharides using 70% ethanol. The supernatant was separated as polyphenols and the portion of the supernatant was used to solvent-solvent partition with hexane, chloroform, and ethyl acetate respectively [3].

In-vitro cytotoxic activity

In-vitro cytotoxic activity was assessed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Based on the results, the potent fraction/s were selected to analyze the apoptosis-related gene expression.

Apoptosis-related gene expression analysis

The human rhabdomyosarcoma (RMS) cell line was obtained from the Department of Biochemistry, Faculty of Medical Sciences, University of Colombo, and the MCF-7 cells were cultured in high glucose DMEM medium supplemented with 10% v/v FBS and 1% penicillin-streptomycin antibiotic solution. The cells were maintained in a 5% CO₂ incubator at 37 °C. The RNA samples of MCF-7 cells treated with hexane fraction of *G. edulis* (15 and 30 µg/mL), standard cycloheximide (30 µg/mL), and untreated cells were extracted using TRIzol reagent. The expression of *p53*, *p21*, *Bax*, and *BCl₂* were analyzed compared to the housekeeping gene β -actin by GoTaq 1-step RT-qPCR system according to the manufacturer's instructions (A 6020, Promega), and relative gene expressions were calculated. Untreated samples are referred to the treated cells with DMEM medium (equal volume as treated cells).

Results and Discussion

The results of the cytotoxicity of the crude methanol extract and fractions of *G.edulis* against the human rhabdomyosarcoma (RMS) cells are shown in Table 1. Based on the results, the potent hexane fraction was selected to analyse the apoptosis related gene expression.

Table 1. Cytotoxicity of crude methanol extract and fractions of *G. edulis*

<i>Extract/Fraction</i>	<i>Cytotoxicity IC₅₀ (µg/mL)</i>
Crude methanol extract	49.86±0.02 ^a
Hexane fraction	32.52±2.15^a
Chloroform fraction	77.07±1.58 ^b
Ethyl acetate fraction	193.99±13.45 ^c
Aqueous fraction	144.09±18.17 ^d
Standard cycloheximide	36.17±1.78 ^a

Results represent means ± standard deviation of triplicate determinations.

The gene expression of *p53*, *p21*, *Bax*, and *Bcl2* showed up-regulated mRNA expression in hexane fraction (15 µg/mL and 30 µg/mL) and standard cycloheximide (30 µg/mL) treated RMS cells. The gene *p53* is a crucial tumor suppressor that regulates the downstream effector *p21*, a potent inhibitor of cell cycle kinases. It also functions as a transcription activator of genes essential for cell cycle arrest, DNA repair, and apoptosis. Besides, *p53* transcriptionally activates pro-apoptotic *Bax* and blocks the function of *Bcl2* [3]. Evaluation of *p53*, *p21*, *Bax*, and *Bcl2* expression is a common approach used for the analysis of apoptosis in response to anticancer compounds/extracts [4].

In the present study, mRNA expression of *p53* and *p21* genes in *G. edulis* hexane fraction treated RMS cells was significantly ($p < 0.05$) up-regulated more than the standard cycloheximide (positive control), which suggest the upstream signaling of the mitochondrial pathway was induced by *G. edulis* hexane fraction. Further, a concentration-dependent mRNA expression of *p53* and *p21* was observed in RMS cells following treatment of *G. edulis* hexane fraction (15 µg/mL and 30 µg/mL), as displayed in Figures 1. The fold change of *p53* and *p21* expression of 15 µg/mL and 30 µg/mL of hexane fraction and standard cycloheximide (30 µg/mL) treated RMS cells were 1.2, 2.13, 0.439 (*p53*) and 0.21, 3.72, 0.06 (*p21*) respectively.

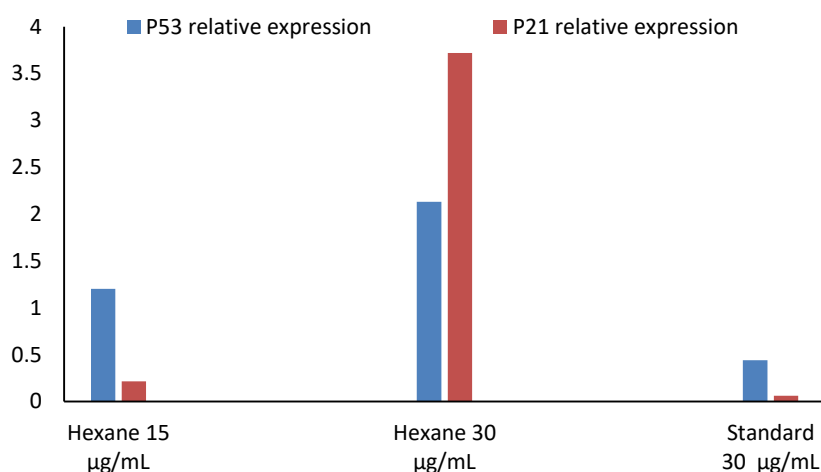


Figure 1. Relative expression of *p53* and *p21* genes in RMS cells

In contrast, hexane fraction (15 and 30 µg/mL) treated RMS cells expressed significantly ($p < 0.05$) different higher levels of *Bax* and lower levels of the *Bcl-2* gene expression as shown in Figure 2. The fold change of *Bax* expression of the

hexane fraction (15 and 30 µg/mL) and standard cycloheximide (30 µg/mL) treated RMS were 0.42, 0.829 and 0.016.

Similarly, the fold change of *Bcl2* expression of hexane fraction (15 and 30 µg/mL) treated RMS cells were 0.119 and 0.098. However, the standard cycloheximide-treated RMS cells expressed a comparatively higher level of *Bcl2* gene than the two concentrations of hexane fraction (Figure 2). The fold changes of *Bcl2* gene expression of standard cycloheximide treated RMS cells were 0.47.

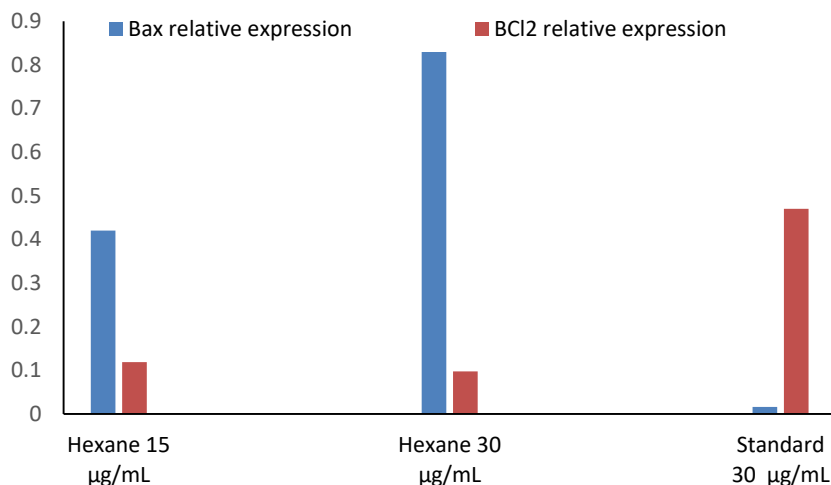


Figure 2. Relative expression of Bax and Bcl₂ genes in RMS cells

Further, the expression level of the *Bax/Bcl-2* ratio can be used to detect the influence of the ability of a cell to respond to an apoptotic signal [5]. As shown in Figure 3, a higher expression ratio of *Bax/Bcl2* was observed in the hexane fraction (30 µg/mL) treated RMS (8.45) cells as shown in table 2.

Table 2. Relative expression of *Bax /Bcl₂* ratio in RMS cells

Extract	Bax/Bcl2 ratio
Hexane 15 µg/mL	3.529411765
Hexane 30 µg/mL	8.459183673
Standard 30 µg/mL	0.034042553

Conclusions and Recommendations

It can be concluded that the hexane fraction of *G. edulis* induced apoptosis in RMS cells mainly via the activation of *P53* and *p21* gene. Hence, hexane fraction of *G. edulis* can be utilized for the development of new drug or supplement to treat the patients suffering from breast adenocarcinoma. Further, it is recommended to isolate the active compounds present in the hexane fraction of *G. edulis*. Further,

it can be recommended to perform a western blot analysis to detect the activation of either the intrinsic or extrinsic pathway of apoptosis

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FOCUS AREA
Mineral Resources

SYNTHESIS AND CHARACTERIZATION OF POLY (ETHYLENE GLYCOL)-GRAFTED GRAPHITE AND GRAPHITE OXIDE AND COMPARISON WITH NATURAL GRAPHITE

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Introduction

Graphite based materials have been one of the most studied materials in the history of humanity due to their remarkable properties such as high thermal, electrical, mechanical, and permeability properties, among others [1]. Further, most of graphite based materials are utilized for different electronic applications as automotive lightweight materials, aeronautics and energy, Li batteries, paints, functional coatings, solar cells, biosensors, membranes, and electronics [2]. In contrast, natural graphite (NG) occurs as a non-functional material since it has a hexagonal layered structure [2] and, hence NG does not directly used for advance applications. Therefore, researchers have given their attention to modify natural graphite using different mechanical or chemical methods [1]. Hence in this study, NG has been modified into two different forms namely poly (ethylene glycol)-grafted graphite (PEG-g-graphite) and graphite oxide (GrO). PEG-g-graphite was synthesized using PEG. As reported, the PEG shows hydrophilic and hydrophobic actions according to its chemical structure [3], therefore, PEG-g-graphite would indicate various interactions with very different components. The GrO could be used for wide-scale chemical interactions due to the presence of the oxygen functionalities such as carbonyl, hydroxyl, epoxy and carboxyl [1]. Additionally, GrO exhibits good hydrophilicity and hence its dispersibility in water is excellent [2]. Therefore, chemically modified graphite would be important to enhance the performance and, to produce advanced applications. The synthesized materials of PEG-g-graphite and GrO were characterized using advanced techniques, such as FTIR, XRD, SEM etc and characteristics were compared with that of the natural graphite material.

Materials and Methods

Materials

RSS-2 (smoked rubber sheet) consisting of a plasticity retention index of 64 was supplied by the Rubber Research Institute of Sri Lanka. PEG with a number-average molecular weight of 4000 g mol⁻¹ was purchased from the local market. Maleic anhydride (MAH) was used as the grafting material and was obtained from

Morex Lanka (Pvt.) Ltd., Sri Lanka. Graphite having a mean particle size of 14 microns was used as a conductive filler and was obtained from Bogala Graphite Lanka PLC., Sri Lanka. N, N-dimethylformamide (DMF) and all rubber compounding ingredients were purchased from local suppliers. Potassium permanganate (KMnO₄), ethanol (C₂H₅OH), hydrochloric acid (HCl), sulfuric acid (H₂SO₄) and hydrogen peroxide (H₂O₂) were purchased from Organic Trading (Pvt) Ltd, Sri Lanka.

Synthesis of PEG-g-graphite

Graphite was added into DMF solution, to form a stable graphite/DMF solution via ultrasonication. Then, the graphite/DMF mixture was heated in the presence of an excess of MAH at 100 °C for 24 hours with dry nitrogen gas allowing it to react with the mixture. Afterwards, PEG was added according to the ratio PEG: MAH, 1:1. The mixture was reacted at 80 °C for 48 hours through continuous mechanical stirring and was poured into a beaker containing ethanol. The mixture was filtered at low pressure and the final product was dried at 50 °C in a vacuum oven.

Synthesis of Permanganate-GrO

GrO was synthesized by Improved Hummers method. In this method, 15 g of graphite was mixed with 45 g of KMnO₄, and diluted slowly in 400 ml of sulfuric acid. The solution was stirred for 3 hours at 50 °C. The oxidation was stopped by adding a mixture of 3 ml of H₂O₂ and 400 g of flake ice, and the solid was filtered off under vacuum. The mixture was washed with 200 mL of distilled water, HCl and ethanol. The resulting filter cake was dried at 60 °C during 1 hour. The product was denoted as Permanganate-GrO [4].

Characterization techniques

Graphite, PEG-g-graphite and GrO were characterized using the x-ray diffractometer (XRD), fourier transform infrared spectroscopy (FTIR), thermogravimetric analyzer (TGA) and scanning electron microscopy (SEM).

Results and Discussion

FTIR analysis was performed to detect the oxygen functional groups attached to the material structure as shown in Figure 1. The absence of peaks in the spectrum of graphite indicates 100% carbon in the structure [4]. According to the FTIR spectrum of PEG-g-graphite, it is shown rather functionality compared to the neat graphite (NG) due to few functional groups containing in the range 1000-2000 cm⁻¹. Further, peaks at 2935 and 2915 cm⁻¹ are assigned to asymmetrical and symmetrical stretching vibrations of C-H bonds, respectively (Figure 1b), which belong to the PEG molecular chain [4]. In addition, a broad peak has appeared in the PEG-g-graphite spectrum at 3340 cm⁻¹ corresponding to the stretching

vibration of O-H groups. Furthermore, the weak peak at 1028 cm⁻¹ is attributed to the stretching vibration of C-O-C bond in the ester groups of PEG-g-graphite owing to the reaction of hydroxyl group with MAH as shown in Figure 1a. When considering GrO, a large number of intense peaks has appeared representing the presence of oxygen groups attached to the structure. GrO shows different peaks relating to OH, C-O-C, C-O, C=O, etc. According to Figure 1, OH stretching vibration peak is observed around 3000 cm⁻¹ and C=O peak is observed at 1700 cm⁻¹. Further, evidence for the presence of the C-O group is observed from the GrO spectrum at 1080 cm⁻¹ [4].

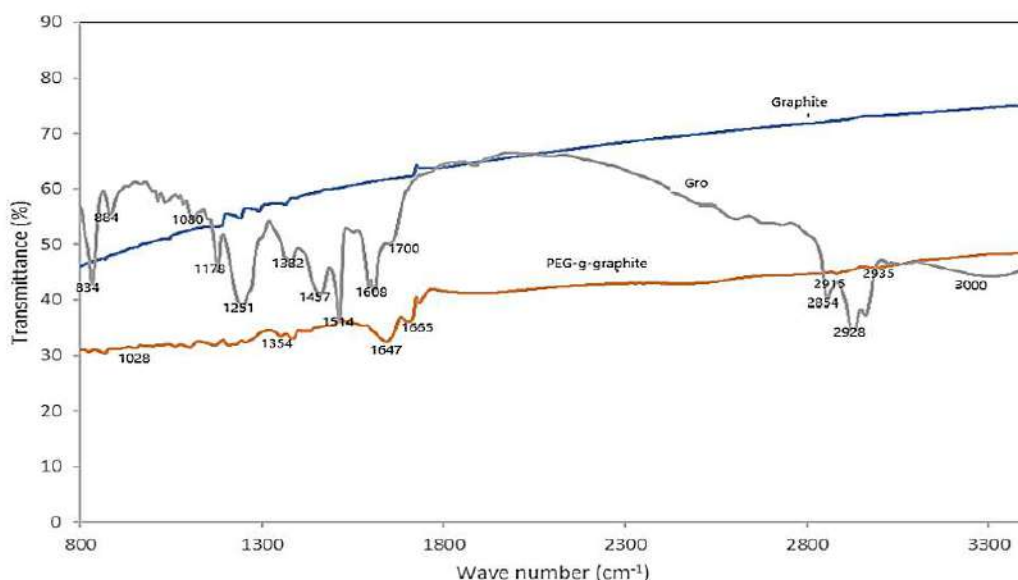


Figure 1. FTIR spectrums of graphite, PEG-g-graphite and graphite oxide

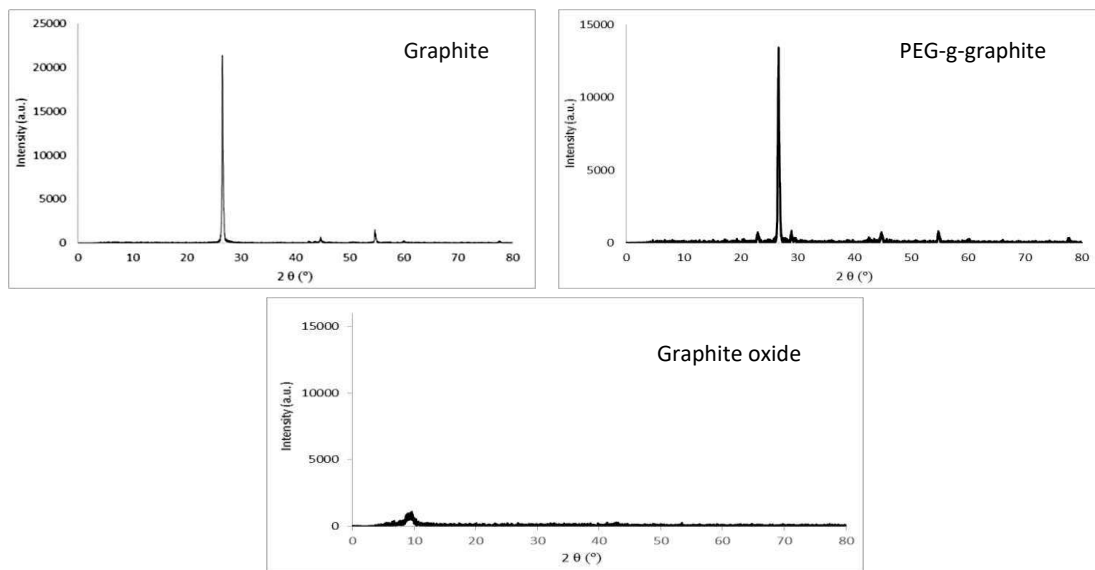


Figure 2. XRD patterns for graphite, PEG-g-graphite and graphite oxide

The TGA curves corresponding to NG, PEG-g-Graphite and graphite oxide are shown in Figure 3. On the basis of the results, graphite exhibited no significant weight loss up to 600 °C and hence, NG confirms high thermal stability. However, two weight loss stages are observed in PEG-g-graphite after its modification. Further, primary weight loss stage is shown in the temperature range of 120–205 °C. The secondary weight loss stage is shown in the temperature range of 205–400 °C, which originates from the grafted PEG chain [5]. Furthermore, different steps can be observed in the TGA curve of GrO as it contains more functional groups. The first step can be observed between 0 and 130 °C and this is due to the loss of water and more thermolabile oxygen functional groups [4]. The second weight loss (130–450 °C) is associated with the removal of the more stable oxygen groups attached to the graphitic structure.

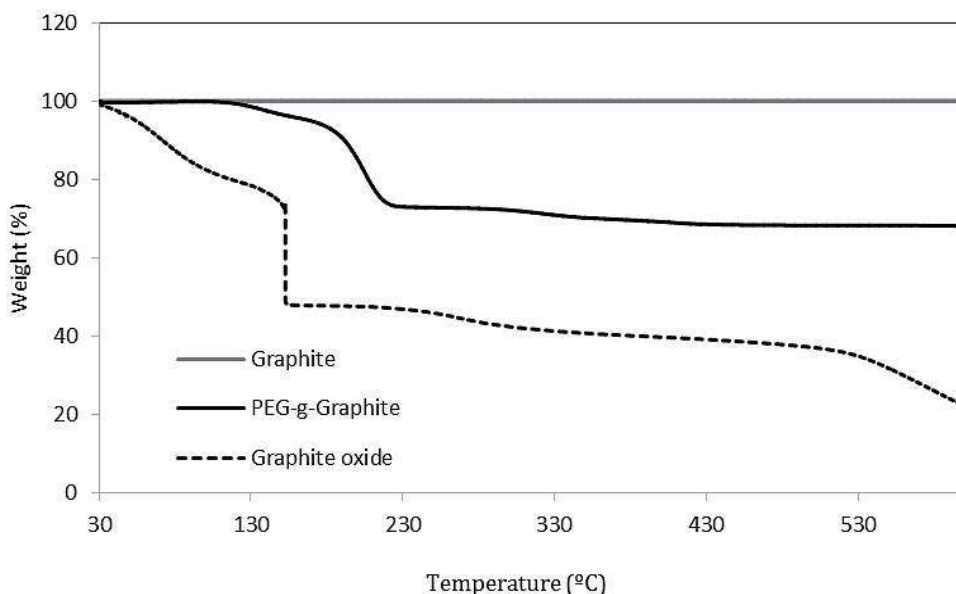


Figure 3. TGA curves of graphite, PEG-g-graphite and graphite oxide

Surface morphologies of graphite, PEG-g-graphite and graphite oxide were studied using SEM as shown in Figure 4. SEM micrograph of the graphite surface (Figure 4(a)) does not show good adhesion between the graphite particles and also, most particles are irregular in shape. However, in PEG-g-graphite (Figure 4(b)), dispersibility and homogeneity of graphite particles have been improved. Furthermore, free volume of the PEG-g-graphite surface is less than that of the NG due to improved surface adhesion among the graphite particles via grafting with PEG. In addition, PEG chains could reduce van der Waals forces between graphite particles. Other than that, micrograph of GrO (Figure 4(c)), shows a fine morphology and hence indicates better homogeneity compared to NG and PEG-g-graphite. Also, it can be seen that graphite particles of GrO are interconnected and uniformly distributed due to presence of a large number of functional groups as indicated by the FTIR spectrum (Figure 1).

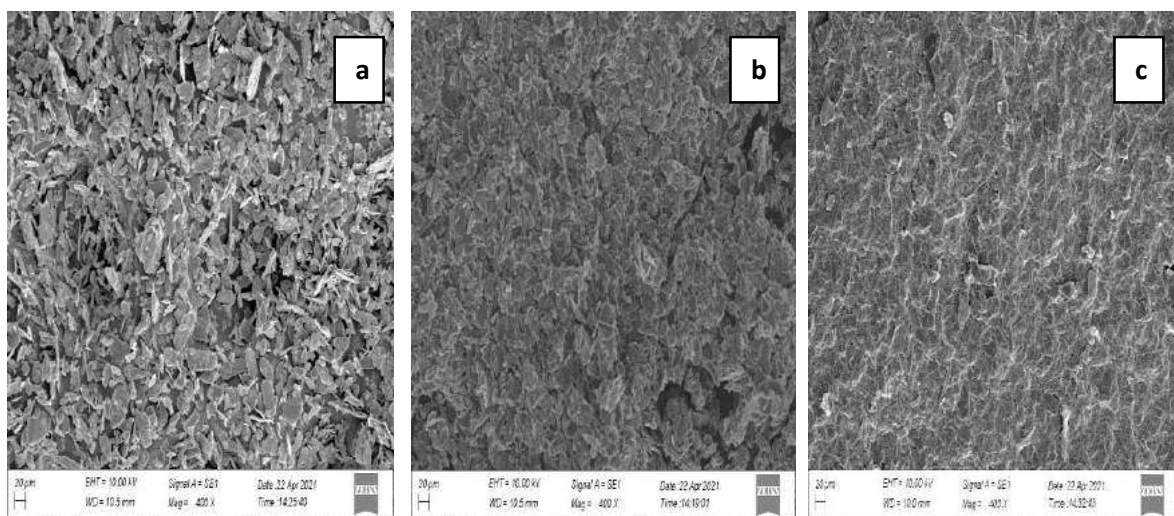


Figure 4. SEM images of surface of (a) graphite and (b) PEG-g-graphite (c) graphite oxide

Conclusions and Recommendations

The aforementioned IR spectra, SEM images, XRD patterns, and TGA data well-demonstrate characterization of PEG-g-graphite and GrO. Further, PEG-g-graphite and GrO indicated the presence of more functional groups and, free volume of the PEG-g-graphite and the GrO surface was less than that of the NG. NG showed better thermal stability than the surface modified graphite materials. However, functional graphite materials would be highly advantageous to combine with other components such as plastic, rubber, fiber, etc.

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FOCUS AREA

Water

WATER QUALITY ESTIMATION IN KIRULAPONE CANAL: APPLICATION OF WEIGHTED ARITHMETIC WATER QUALITY INDEX METHOD

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Introduction

Water is one of the essential resources on which human survival and settlement are based. Rivers, glaciers, rainwater, groundwater are the forms of water on the earth. The degradation of these natural water resources and the management of existing freshwater is becoming more difficult for various reasons, including climate change, geology, terrain, and soil type [1].

Aside from the natural characteristics of these natural water resources, the key element affecting water quality and management of these natural water resources is anthropogenic influence. As a result, managing water quality and availability of freshwater has made it more difficult for water service providers, particularly in developing countries. These activities, in some way, generate toxins that wind up in rivers, streams, and oceans via runoff and effluent disposal. Climate change and land usage are two elements that will directly impact the water bodies available for human and agricultural use [2]. Improper land use causes soil erosion, which increases the mobilization of suspended particles in receiving waterways via runoff. Sri Lanka has 103 natural river basins with a total length of around 4,500 kilometers. However, the increased demand for urban activities due to fast population expansion has impacted the water quality of these river bodies. Several researchers have determined that the decline in river water quality is caused by point sources of pollution such as direct discharges from various land-use types such as residential, industrial, and agricultural, and non-point sources of pollutants such as urban stormwater runoff [3].

Colombo is the administrative and economic capital of the country. The Colombo metropolitan region is covered with artificial infrastructures and natural resources. Within the Colombo metropolitan region, 37 canals have been identified. These canals are all linked together to form a canal network. Several pollution source points have also been identified within the canal network.

As a densely populated area, Colombo generates a large amount of wastewater discharged into the canal without a proper piped collection system. As a result, the bodies of water may become polluted. Furthermore, this behavior causes severe water pollution and enormous economic and social costs.

Several studies on water quality assessment in the Colombo basin were conducted, but no researchers monitored the Kirulapone canal in Colombo. Thus the study can provide comprehensive water quality details for the Kirulapone canal, improving watershed management. It can also serve as a resource for the researcher in future studies. Hence, this study aims to assess the temporal variation of the water quality index of the Kirulapone canal in 2015 and 2020, using the weighted arithmetic water quality index method.

Materials and Methods

The Kirulapone canal has been located in the Colombo district, among the rapidly urbanized area. The sample was collected in the midpoint (6°53'16.05"N 79°52'56.13"E) of the Kirulapone canal using a sterile 250 mL Schott bottle and the handheld water quality meter. The laboratory analysis has been done for data formation of water quality parameters at the Kirulapone canal.

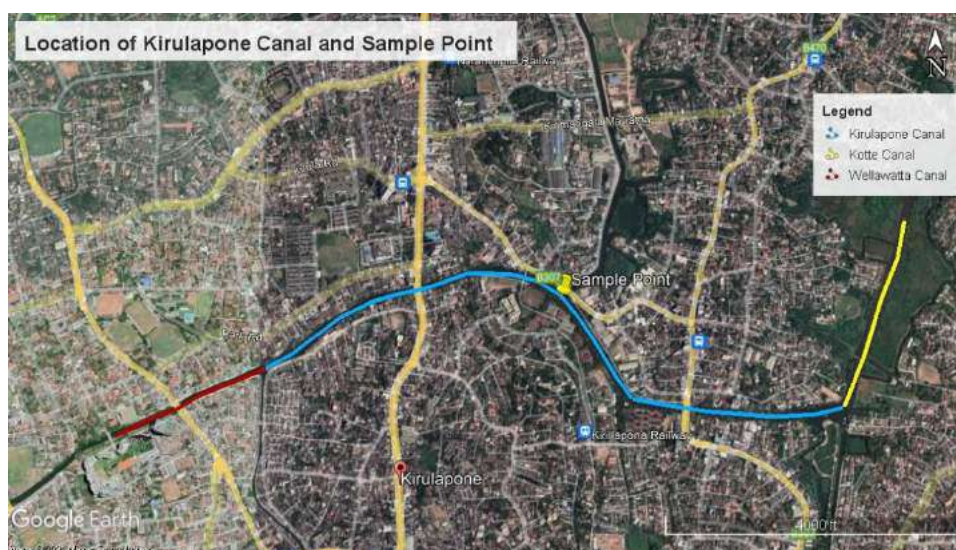


Figure 1. Sample collected location at Kirulapone canal

Water quality parameters such as pH, electrical conductivity (EC), dissolved oxygen concentration (DO), ammonia concentration (NH_3), nitrate concentration (NO_3^-), phosphate concentration (PO_4^{3-}), chemical oxygen demand (COD), total dissolved solids (TDS), and temperature were measured to assess the water quality of the Kirulapone canal during 2015 and 2020 [4].

It was calculated using the Sri Lankan central environmental authority's standard. The water quality index was calculated using the weighted arithmetic index method [5].

The water quality index was calculated by the following equation,

$$W_i = K/S_i$$

$$Q_i = \frac{[(V_i - V_o)]}{[(S_i - V_o)]} * 100$$

$$\text{Overall WQI} = \frac{\sum W_i Q_i}{\sum W_i}$$

Where,

K: Proportionality constant

S_i: Standard desirable value of the ith parameters on the summation of all selected parameters unit weight factors W_i = 1 (unity)

V_i: Mean concentration of the ith parameters

V_o: Actual values of the parameters in pure water (V_o = 0 for most of the water parameters except pH = 7.0 and DO = 14.6 mg/L)

Table 1. Water quality rating as per the weighted arithmetic water quality index method

Rating of Water Quality	WQI value
Excellent water Quality	0 to 25
Good Water Quality	26 to 50
Poor Water Quality	51 to 75
Very Poor Water Quality	76 to 100
Unsuitable for drinking purposes	Above 100

Results and Discussion

According to the analysis, the average pH value in 2015 was 6.48, and in 2020 was 6.41. The pH standard in Sri Lanka runs from 6 to 8.5, which is adequate to support human and agricultural demands, and the average value of the canal was measured within the standard level. In 2015, the average EC was 0.21, and in 2020, it was 0.30. In Sri Lanka, the conductivity standard for irrigation is 0.7 dS/cm. In both 2015 and 2020, the conductivity of the Kirulapone Canal water was less than 0.7 dS/cm. DO had a mean value of 5.01 in 2015 and 4.2 in 2020. The DO guideline is 3 mg/L, yet the canal water was considerably polluted in 2015 and 2020. The standard ammonia concentration is 1 mg/L. The level of ammonia in 2015 was less than 1 mg/L, but by 2020 it had risen to 1.73 mg/L. As a result, the water will be unfit for aquatic species in 2020.

The nitrate standard in Sri Lanka is 5 mg/L, and at the Kirulapone canal, it was 0.11 in 2015 and 0.44 in 2020. The canal's nitrate level was found to be within acceptable limits. The phosphate standard in Sri Lanka is 0.7 mg/L. The phosphate level was 0.10 in 2015 and 0.55 in 2020, both within acceptable limits. COD levels in 2015 were 28.00 mg/L, while in 2020, they were 26.08 mg/L, which was more

than the standard level (above 15 mg/L). In addition, in 2015 and 2020, the TDS was recorded as below the standard level.

Furthermore, the Kirulapone canal's water quality characteristics were used to determine the water quality standard. The weighted arithmetic index approach is used to classify water quality based on purity. The following are the findings of the analysis:

Table 2. Metrics of WQI 2015 in Kirulapone canal (location: 6°53'16.05"N 79°52'56.13"E)

Water Quality Parameters	Sn	Wn	Vi	Qi	WiQi
pH	8.5	0.026	6.48	76.24	1.96
EC	0.7	0.312	0.21	30.00	9.36
DO	3.0	0.073	5.01	167.00	12.16
Ammonia	1.0	0.218	0.08	8.00	1.75
Nitrate	5.0	0.044	0.11	2.20	0.10
Phosphate	0.7	0.312	0.10	14.29	4.46
COD	15.0	0.015	28.00	186.67	2.72
TDS	0.5	0.437	0.10	20.00	8.74

Sn: Standard Value; Wn: Unit Weight; Vi: Mean Value; Qi: Quality Rating; WiQi: Water Quality Index

Table 3. Metrics of WQI 2020 in Kirulapone canal (location: 6°53'16.05"N 79°52'56.13"E)

Water Quality Parameters	Sn	Wn	Vi	Qi	WiQi
pH	8.5	0.026	6.41	75.41	1.94
EC	0.7	0.312	0.30	42.86	13.38
DO	3.0	0.073	4.21	140.33	10.22
Ammonia	1.0	0.218	1.73	173.00	37.80
Nitrate	5.0	0.044	0.44	8.80	0.39
Phosphate	0.7	0.312	0.55	78.57	24.53
COD	15.0	0.015	26.08	173.87	2.53
TDS	0.5	0.437	0.20	40.00	17.48

Sn: Standard Value; Wn: Unit Weight; Vi: Mean Value; Qi: Quality Rating; WiQi: Water Quality Index

According to the Weighted Arithmetic WQI, the total water quality index for 2015 is 41.248, indicating good water quality. In 2020, the complete water quality index was 108.257, indicating that the water is unfit for consumption. The results demonstrate that the mean value of water quality parameters such as ammonia, nitrate, phosphate, and TDS was higher in 2020 than in 2015. The volume of waste effluents dumped into the Kirulapone canal has increased due to the rapid construction growth along the canal's sides. The majority of sewage from the neighborhood is discharged directly into the canal. It was the cause of a drop in water quality in 2020 compared to 2015.

Conclusion and Recommendation

The research was utilized to examine the limited amount of data available between 2015 and 2020. Different trends of water quality metrics were recorded. The computed water quality index values of the Kirulapone canal in 2015 and 2020 are 41.248 and 108.257, respectively. The results showed that the water quality was good in 2015, but it was no longer appropriate for drinking in 2020. According to the canal's location, the key deciding element of water quality is urbanization. Due to population density and development, various direct pollution sources have been detected within the Kirulapone canal.

Identifying all direct pollution sources, managing those locations, and establishing alternate drainage systems to remove wastewater from residences and companies is the first and best technique for mitigating water pollution in the Kirulapone canal. Implementation of rules and regulations to prevent human activities that affect water pollution also helps to protect the water bodies.

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GROUNDWATER DEPLETION IMPACT ON THE SEA-LEVEL RISE AROUND THE JAFFNA PENINSULA, SRI LANKA

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Introduction

With the impact of climate change, sea level rise (SLR) has created a prominent danger for the small islands and coastal cities. Hence SLR is not uniform for all the regions, it is behaving unpredictably at regional and global scales. It depends on the atmospheric and ocean circulation, the gravitational field of the earth, thermodynamic expansion of ocean water, ice sheet dynamics. Global total sea level rise is mainly determined by two factors such as thermal expansion of ocean due to warming of ocean and, melting of land ice and melt water directed to oceans. Heat trapped in the earth climate system is absorbed by the ocean water cause the expansion of water. Sri Lanka is also an island especially vulnerable to climate change and its impact. When it comes to regional Relative sea level rise it can be affected by multiple parameters through space and time. Beside this ground water from aquifers consumed for the human needs end up in the ocean adding to relative sea level rise. Jaffna peninsula is situated in the north of the country facing observable impacts from the SLR. The coastal belt of Jaffna is estimated to be 160 km and total land mass is span over 1000 km² [1]. About 30% of the land is under 1 m mean above-ground sea level. Although the sea-level rise was predicted as 3.4 mmyr⁻¹ (with global warming, some countries face greater danger from uneven SLR) [2]. The sea-level rise and frequent coastal floods are also emerging issues in all parts of the coastal zones. In this perception Northern part of Sri Lanka receives its rainfall from second inter monsoon prevails from September to November and northeast monsoon which prevails from December to February are the only option for groundwater replenishment. The Jaffna Peninsula has four main aquifer systems, namely Chunnakam (Valikamam area), Thenmaratchi, Vadamaratchi and Kayts, which have appropriate aquifer properties for groundwater storage and discharge. The annual rainfall of the region ranges from 600-1400 mmyr⁻¹. Jaffna population solely depends on rain fed groundwater aquifer resources since ancient times. It is important to notice excess extraction of the groundwater in the coastal cities makes it worse on the coastal tidal flooding and sea-level rise [3]. As the demand for water is increases for agriculture, intensive irrigation, dense population and higher organic fertilizer usage simultaneously increase the saltwater lens to move upward and at the same time subsidence of the land. This initiates the inundation of the coastal cities in the northern part of Sri Lanka and the deterioration of water quality. The

objective of the study was to appraise the impact of groundwater depletion in the sealevel rise and coastal flooding events in the Jaffna peninsula for the present and future.

Materials and Methods

Data

Future sea-level inundation was predicted considering the sea-level trends based on tidal gauge data and high-resolution surface elevation data. We assessed the NASA sea level anomaly data for the Jaffna region and ice sheet fingerprint data for Colombo Sri Lanka. Sealevel anomaly data for the Jaffna peninsula region which spreads within the coordinates of 8.4485 N, 79.0488 E to 10.1052 N, 81.6284 E was assessed from February 2006 to January 2019. Rainfall data for the period of last two decades for the Jaffna region was obtained from NASA IMERG version 06 satellite gauge data. We searched the past literature for possible area inundation and possible population impact.

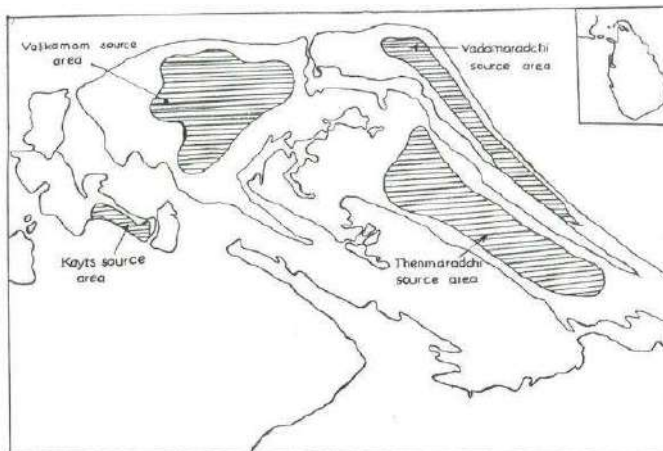


Figure 1. Groundwater aquifers in Jaffna Peninsula (Source: [8])

Methodology

An elevation map of the Jaffna peninsula was obtained from the webpage topographic.com. Sea level anomalies Sea level rise regarding thermal expansion and ice sheet fingerprint were obtained from the NASA sea level data store. We have added on all the ice sheet fingerprint glacier melting contributions to the SLR in the regions. Also, mean sea level rise for the worst-case scenario SSP8 for Sri Lanka was obtained from the same data store. From that, we had calculated the rate of land submerging due to the over-extraction of groundwater. Also, we used the same data source to assess the impact area from the future SLR. SLR in the region is given by the following formula.

Sealevel rise in Jaffna peninsula = Sea level change due to over extraction of groundwater + Thermal expansion of seawater + Ice sheet fingerprint

By comparing the aquifer recharging potential from the trend of seasonal rainfalls including pre-monsoon and monsoon rain fed water through the IMERG image obtained from the GIOVANNI site.

Results and Discussion

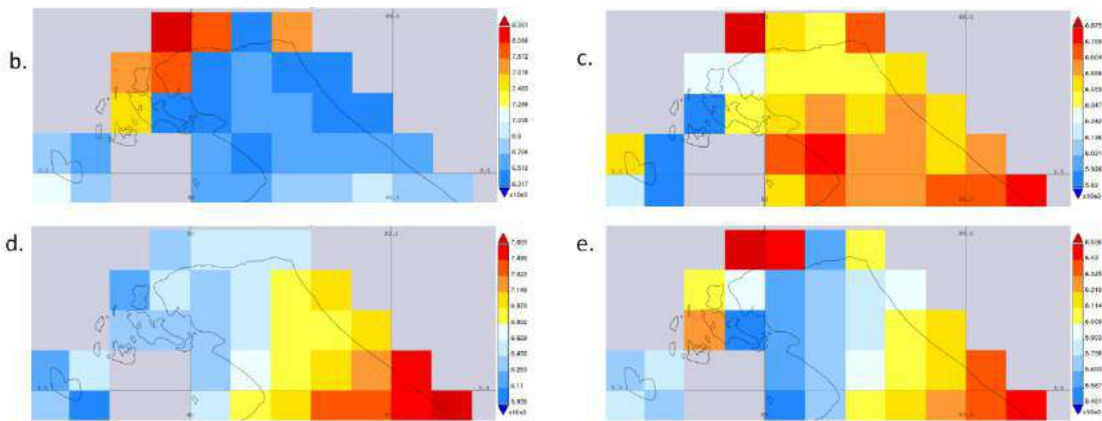


Figure 2. b, c, d, e shows the mean precipitation for a consecutive five-year period from 2001 to 2020 (b. 2001 to 2005, c. 2006 to 2010, d. 2011 to 2015, e. 2016 to 2020) over the Jaffna region

Figure 2 shows the aquifer recharge was significantly impacted by the reduction in rainfall in the last two decades.

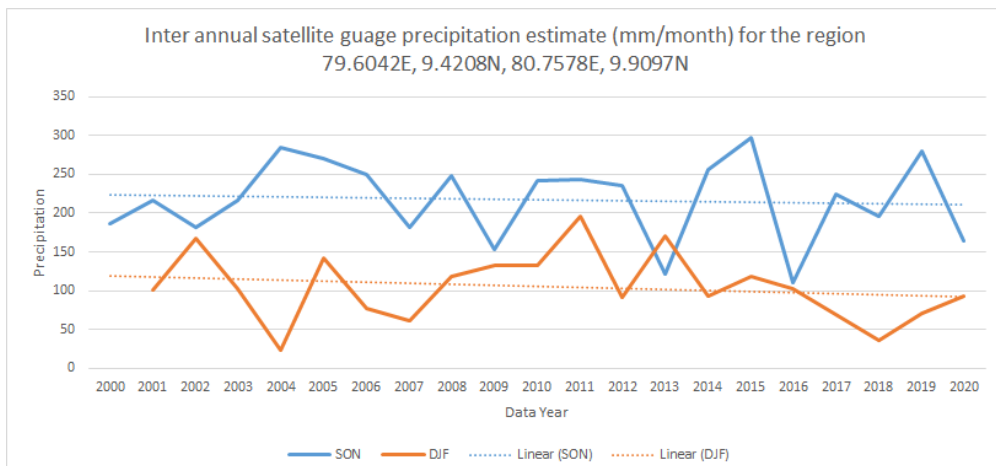


Figure 3. Seasonal Precipitation mm/month estimate for second inter monsoon shown black colour and Northeast monsoon shown by the blue colour from 2000 to 2020

The trend lines for the second inter monsoon and Northeast monsoon shows decreasing rainfall in the Jaffna region for the major contributor for the ground water replenishment.

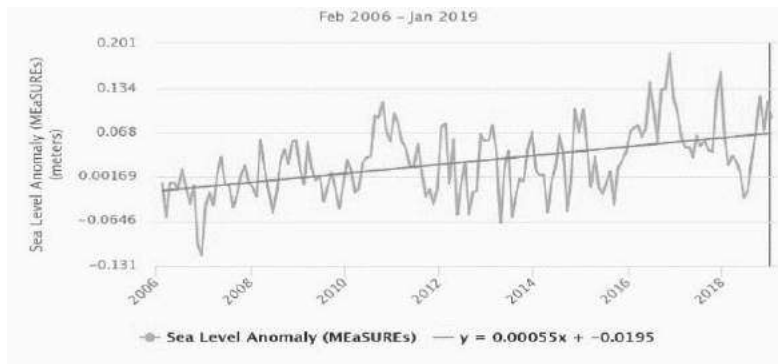


Figure 4. Seal level anomaly measure from Feb 2006 – Jan 2019

The trend from the figure 4 shows the annual increase in sea level at a rate of 5.5 mmyr^{-1} around the Jaffna peninsula [5].

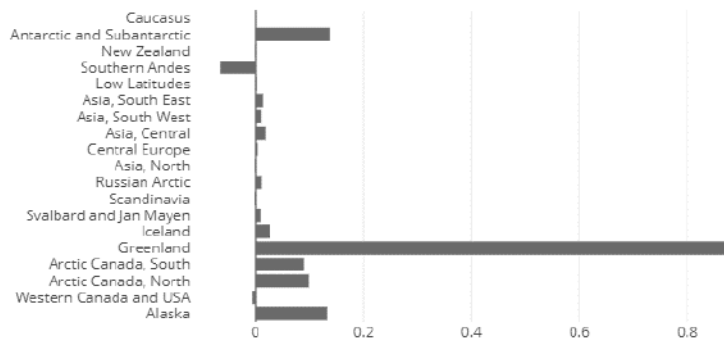


Figure 5. ice sheet fingerprinting shows the contribution from ice sheet melting for SLR in the regions [5]

Combining all the inputs from the glacier melting, impact from ice sheet fingerprint on sea level rise is 2.08 mmyr^{-1} . Note that the most of the glacier melt impact is

from the Greenland continental shelf.

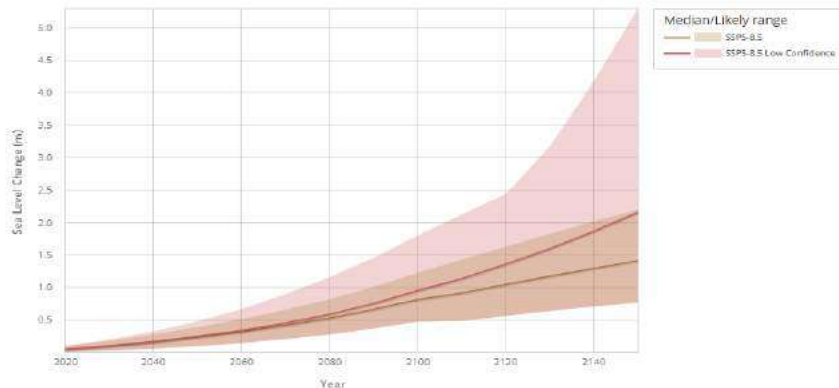


Figure 6. Mean sea level change for the Shared socioeconomic pathway scenarios SSP-8.5 and SSP-8.5 low confidence for Sri Lanka

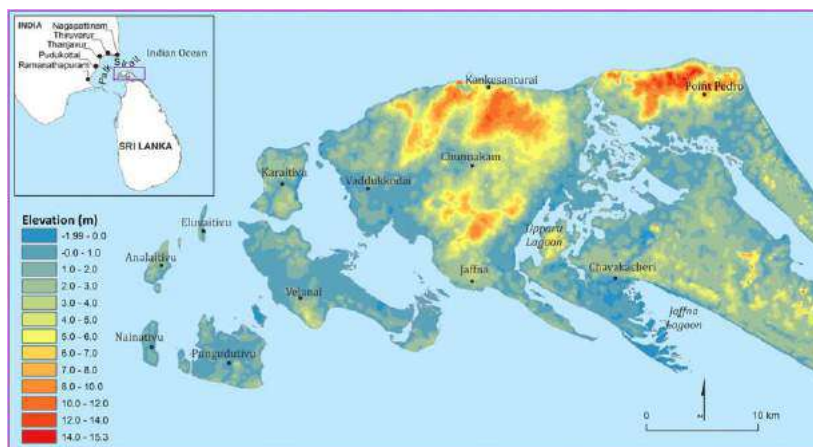


Figure 7. Highly vulnerable region for SLR crisis which accounts for nearly 50 % of the land area under the 1-3 m altitudes [4]

Under the Intergovernmental Panel on Climate Change Representative Concentration Pathway (RCP) scenarios by 2100, the possible land area inundated by the sea level rise is 49.1% low-end scenario (RCP2.6), 58.5% under the high-end scenario (RCP8.5) [4]. For each worst-case scenario of SSP5, the mean sea level is rise by nearly 1 m in 2100. If this rate continues probably the 7-8 km from the coastal zones which is between 306.9 to 365.63 Km² is under SLR threat. Out of the total area of 1025 km² of Jaffna peninsula, 306.9 to 365.63 km² are at high risk that will be impacted from coastal flooding and salinity changes, where mean above-ground sea level is likely within 1m. The results show the annual increase in the SLR is 5.5 mmyr⁻¹, which is greater than the annual average global SLR of 3.4 mmyr⁻¹. Ice sheet fingerprint contributes to the annual SLR of 2.08 mmyr⁻¹. Sea level rising rate around Colombo is where over extraction is not an option is 3.4 mmyr⁻¹ [5].

Conclusions and Recommendations

It is foreseeable that the SLR around Sri Lanka from thermal expansion of ocean water is about 1.32 mm yr^{-1} and over extraction of groundwater account for 2.1 mm yr^{-1} . Salinity changes of freshwater across the northern part of Sri Lanka are perceptible. There is a significant decline in ground water recharge by the pre-monsoon and monsoon precipitation for the Jaffna region. The reduction of rain fed recharging ability of groundwater is decreasing substantially. Therefore, with increasing population and continuous over extraction, submersion of land is unavoidable. This indicates nearly 49.1% to 58.25% of the region is vulnerable to sealevel rise which is about 7-8 km within the coastal area. Also, the area inundated by the seawater creates dire consequences on crop cultivation which is a main economic activity in the region. It is often required strategies for sustainable integrated water management of water and developing possible alternatives to the ground water to meet this danger in the near future by nearly 0.6 million people.

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FACTORS INFLUENCING RUBBER SMALLHOLDERS' BEHAVIOUR IN ADOPTION OF RUBBER PROCESSING TECHNOLOGIES IN MONARAGALA DISTRICT

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Abstract

This study was carried out to examine factors influencing the behaviour of smallholders in adoption of Processing Practices of Rubber Ribbed Smoked Sheets (PPRRSS) introduced to Monaragala by the Rubber Research Institute of Sri Lanka (RRISL) to reduce the cost of production and also to find out the strategies for improvement of adoption. The data were collected employing a self-administrated survey, from 361 rubber smallholders who had been making rubber sheets of Monaragala in 2020, using stratified random sampling. The conceptual model was developed based on the decomposed theory of planned behavior. The variables were measured with the use of validated items. The responses of rubber smallholders for items were captured on a Five-point Likert scale. The conceptualized model was empirically tested using partial least square structural equation modeling by bootstrapping procedure using the SMART- PLS 3.2 software. The composite six-predictor conceptual

framework and structural model were validly and reliably capable of explaining 77% the variance in the adoption of PPRSS by rubber smallholders. Adoption of PPRSS by rubber smallholders was positively and significantly correlated with the behavioural factors of behavioral intention and perceived behavioral control, while perceived behavioral control under adoption was the most significant influential factor. There were positive significant relationships; compatibility and attitude, relative advantage and attitude, perceived usefulness and attitude, perceived ease of use and attitude. The perceived usefulness, out of the four components compatibility had the most significant effect on attitude. These findings can help boost the behavioral intention of rubber smallholders in Monaragala in using PPRSS, especially by improving the aspects of facilitating condition and subjective norms.

Keywords: Adoption, Decomposed theory of planned behavior, Rubber smallholders

I. INTRODUCTION

Rubber (*Hevea brasiliensis*) sector is contributed 0.2% to the GDP in 2020 [1]. The smallholder rubber sector is considered as the most dynamic segment of the rubber sector as it represents 59% of the total rubber extent of Sri Lanka and contributed 48% to national rubber production in 2019. At present, the total extent of rubber smallholdings in Monaragala is about 5,087ha (9,415 number of holdings) and Monaragala is the fifth rubber growing District according to the land extent under rubber cultivation in Sri Lanka [2]. The Processing Practices of Rubber Ribbed Smoked Sheets (PPRRSS) were introduced to Monaragala area by the Rubber Research Institute of Sri Lanka (RRISL) to reduce the cost of production [3]. The statistics for Monaragala District on level of adoption of practices in PPRSS is very low and not at satisfactory level [4]. The previous studies do not specifically focus on PPRSS in Monaragala [4]. Therefore, this study examined the behavioral factors that influence adoption of PPRSS by Rubber Smallholders (RSs) in Monaragala. The findings will assist in the designing of more effective policy instruments to remove Adoption (ADN) barriers of PPRSS in Monaragala, and it can have a greater impact on the economic development of the country.

II. MATERIALS AND METHODS

Rubber Smallholders (RSs) (297) who have been producing Ribbed Smoked Sheets (RSSs) were selected using the stratified random sampling technique, based on geographical distribution of them in all rubber growing DS divisions (8) in Monaragala (representing 23% of the population at 90% CI). Both primary and

secondary data were collected for this study in 2020. The cross-sectional pre-tested questionnaire survey was carried out. The questionnaire for RSs had both key socio-economic factors and behavioral factors measuring items. In an effort to understand intentions of RSs to adapt to PPRSS, the Decomposed Theory of Planned Behaviour (DTPB) was applied as a theoretical framework [6]. The DTPB focuses on the direct measures of attitude, subjective norms, and perceived behavioural control to predict behavioural intention which in turn predicts one's behaviour [7]. Relative Advantage (RA) refers to the degree to which an innovation provides benefits which replace those of its precursor and may incorporate factors such as economic benefits, image, enhancement, convenience and satisfaction. RA is an important factor in determining attitude of innovations [8]. In this study, RA refers to financial advantages of PPRSS. Therefore, the hypothesis was proposed as an alternate hypothesis, as "perceived RA towards the adoption of PPRSS positively affects attitude of RSs" (H_{RA}). According to Rogers [6], Compatibility (CO) is the degree to which an innovation is perceived being consistent with the existing values, past experiences and need of potential adopters. In this study, CO refers to the extent to which RSs believe that application of PPRSS would be compatible with their rubber farming practices. Therefore, the hypothesis was proposed as the alternate hypothesis, as "perceived CO towards the adoption of PPRSS positively (H_{CO}). Perceived Usefulness (PU) is defined as a person's subjective evaluation of the extent of using a system which would enhance the productivity [9]. In the context of PPRSS, PU would be the degree to which an

individual view that PPRSS would result in more productivity than previous PPRSS. Therefore, the hypothesis was proposed as the alternate hypothesis, as “PU towards the adoption of PPRSS positively affects attitude RSs” (H_{PU}). Perceived Ease of Use (PEU) is defined as the expectation by an individual of the degree to which the target system will be free from effort [9] [8]. In this study, PEU refers to the level of the easiness of using PPRSS introduced by the RRISL. Therefore, the hypothesis was proposed as an alternate hypothesis, as “PEU towards the adoption of PPRSS positively affects attitude of RSs” (H_{PEU}).

According to Rogers [8] Attitude (ATD) refers to general feeling of an individual on favourableness or unfavourableness towards using innovation. ATD is linked to behavioural intention as individual forms psychological intentions to perform behaviours toward which they have positive feeling. In this study, ATD refers to the feeling of RSs about using PPRSS in their rubber cultivation. Accordingly, the hypotheses were proposed as the alternate hypothesis, as “ATD toward the ADN of PPRSS positively affects their BI of RSs” (H_{ATD}). Perceived Behavioural Control (PBC) is defined as the level of confidence of an individual about their ability to perform the behaviour based on the difficulty or ease as they perceive on its performance as it relates to difficulties or facilitators. It reflects the beliefs regarding access to the resources and opportunities needed to affect a behaviour [7]. In this study, PBC refers to adequate level of knowledge and skill of PPRSS. Therefore, the hypothesis proposed as the alternate hypothesis, as “PBC of RSs positively affects on ADN of PPRSS” (H_{PBC}). Facilitating Condition (FC) is defined, as the environmental factors that

influence an individual’s desire to perform a task. FC reflects the availability of resources/inputs (time, money, and other specialized resources) which are needed to engage in a behaviour [6]. In this study, availability of inputs and advisory contacts done by extension officers and availability of training programmes refers to FC for the BI of PPRSS. Therefore, the following hypothesis was proposed as the alternate hypothesis: FC towards the use of PPRSS positively affects PBC of RSs (H_{FC}).

Subjective Norms (SN) is defined as “the person’s perception that most people who are important to him think he should, or should not, perform the behaviour in question”. The determinant of SN is the sum of normative beliefs which reflects the perceived behavioural views or expectations of important referent individuals or groups. The adopter’s family members, friends, and colleagues are groups that will potentially influence SN [7]. In this study, SN towards ADN of PPRSS refers to the influence of extension officers, RSs and mass media. Therefore, the following hypothesis was proposed as the alternate hypothesis; The SN toward the ADN of PPRSS positively affect their BI of RSs (H_{SN}). Behavioural Intention (BI) as a person’s subjective probability in performing certain behaviour. In this study, BI refers to the intention to apply the PPRSS in the next season, on a regular basis and to strongly recommend it to others too. Therefore, the following hypothesis was proposed as the alternate hypothesis; the BI of RSs positively effects on adoption of PPRSS (H_{BI}). Rogers [8] defined Adoption (ADN) as a decision to make full use of a new idea as the best course of action available. Accordingly, the process of ADN or innovation decision is a

psychological process in which an individual move from awareness, interest, evaluation and to trial and finally either to adopt or to reject the practice. In this context, ADN refers to utilization and application of PPRSS recommended by the RRISL.

Ajzen [7], Hagger and Chatzisarantis, 2005, were reviewed to generate an initial list of items. Scales containing multiple items were developed to measure each of the following variables namely, BI, ATD, PBC, SN, PEU, RA, CO, FC and PU were measured by sets of items using a five-point Likert scale that ranged from 'strongly disagree' for which it was given 1 to 'strongly agree' for which it was given 5, indicating the degree to which they agreed with the set of statements. PU, PEU, CO, RA, SN, PBC and ATD were measured two items while, BI, FC and ADN were measured with three items. Pre-test interviews were conducted with RSs to assess the instrument's clarity and question wordings of proposed items.

Two-stage model-building process was applied for testing both measurement and structural models [10]. The Cronbach's alpha (α), Composite Reliability (CR) and Factor Outer Loadings (FOL) were assessed to measure the reliability, validity and internal consistency of items respectively, for covering the Convergence Validity (CV). The Average Variance Extracted (AVE) was assessed to measure Discriminant Validity (DV) of items. The Heterotrait-Monotrait ratio of correlations was used to assess DV of questionnaire. The measurement model was estimated using Confirmatory Factor Analysis (CFA) to test reliability and validity of the measurement model [10].

After assessing the reliability and validity criteria for all reflective measurement of the

research model and ensuring the integrity of the research data, SMART-PLS Algorithm was applied after determining 300 maximum iterations with stop criterion of 7 using path scheme to maximize the R^2 value for the model endogenous latent variables. For predictive purposes, Partial Least Square Approach for the Structural Equation Modelling (PLS-SEM) was applied. The coefficient of determination (R^2), effect size (f^2), Stone-Geisser (Predictive relevance) index (Q^2) and the path coefficients (β) were assessed using the blindfolding procedure which necessitates the predictive capacity measurements as suggested by Hair *et al.*, [10]. The cross-validated redundancy method was used to measure the Q^2 by using a blindfolding procedure as recommended. The Variance Inflation Factor (VIF) was applied to assess the Multi-collinearity and inter-correlations among the independent constructs within the structural model (inner model). Both the model goodness-of-fit indices; (SRMR) and Normed Fit Index (NFI) of the structural model were assessed to examine the model fit [10].

The Bootstrapping procedure was applied to examine β significance which is a variance based method used to estimate structural equation models, using Smart PLS 3.1. The t-statistics were used to test the statistical significance of both the indicators (outer model) and the structural model constructs (inner model). Two-tailed t-test of significance at 5% level was carried out, with t-statistic values larger than 1.96 indicating significance of the structural path significance tests. The results were interpreted with standardized β and coefficients of determination (R^2), with the bootstrap samples set at 5000 and the

standard error at a 95% confidence level as suggested by Hair *et al* [5].

III. RESULTS AND DISCUSSION

A. Test of measurement model and assessment of the structural model

The α values ranged from 0.79 to 0.98, which were above the acceptable threshold 0.70. The FL for all items exceeds the recommended level of 0.6. CR values of all items exceeded recommended level of 0.7 (range from 0.72 to 0.97). According to the α (>0.07), CR (>0.7) and FL (>0.6) which were greater than standard values, indicating the questionnaire had high reliability, validity and internal consistency, also indicate that the items of questionnaire had satisfactory CV. The AVE were in the range between 0.548 and 0.932 and AVE of all items exceeded recommended level of 0.5. DV was well established across all the constructs as no item is cross-loaded higher on another construct than on its own construct. In summary, the measurement model demonstrated adequate communality, reliability, CV, DV and fitness of model (Hair *et al.*, 2017). R^2 with a value of 0.68, 0.33 and 0.19 is considered substantial, moderate and low, respectively (Chin, 2010). R^2 of ATD towards BI, BI towards to ADN and PBC towards to ADN were 0.722 (Substantial), 0.679 (Substantial) and 0.426 (Moderate), respectively. The R^2 of the structural model of this study was 0.775 and it was considered substantial in line with recommended value. The four exogenous variables (ATD, BI, PBC, SN) explain 77% of the ADN of PPRSS by RSs. Effect size (f^2) of 0.03, 0.16, and 0.36 indicates small, medium, and large effect, respectively [11]. Four hypothesis (H_{ATD} , H_{PBC} , H_{FC} and H_{BI}) are in the large effect category, whilst the rest

(H_{RA} , H_{CO} , H_{PU} , H_{PEU} and H_{BI}) are medium effect size. Since Q^2 values for all latent variables (ADN = 0.763, ATD = 0.610, BI = 0.459 and PBC = 0.314) of the inner model were greater than zero, the path model had a favourable strong predictive relevance. All of VIF values for all independent latent variables were less than five and fulfil the requirement of data according to the Multicollinearity test and so there was no collinearity problem according Hair [10]. The SRMR value for the study model was 0.074 and NFI value was approximately 0.8. It could be concluded that both indices represent acceptable fit for the research model and a good fit [10]. The previous analytical and statistical outcomes gave adequate answers to test research hypotheses.

B. Hypotheses testing

The results showed that RA, had positive effect on attitude towards adoption of SDTS and intention to use PPRSS for adopters (H_{RA}) ($\beta = 0.115$, t-value = 5.224, $p < 0.05$). CO had positive effects on attitude towards adoption of PPRSS (H_{CO}) ($\beta = 0.368$, t-value = 6.091, $p < 0.01$). It reveals that RSs perceive practicing the PPRSS being consistent with their existing cultural practices of RF, lifestyle and past experience. PU had positive effects on attitude toward PPRSS (H_{PU}) ($\beta = 0.354$, t-value = 5.617, $p < 0.05$). PEU had positive effects on attitude towards PPRSS adoption (H_{PEU}) ($\beta = 0.355$, t-value = 5.612, $p < 0.01$). The importance of characteristics (RA and CO) of innovation on adoption is emphasized (Rogers, 1995). However, effects of CO on ATD towards PPRSS are comparatively high.

ATD had significant positive relationship with behavioral intention and thus

supported H_{ATD} ($\beta = 0.324$, t -value = 3.872, $p < 0.01$). ATD was also the most influential factor that predicts intention of RSs to use PPRSS in Monaragala. This result implies that if RSs have a positive attitude, they will certainly be more attracted to use SDTS. ATD is considered as a powerful factor that motivates to develop a positive intention. The H_{SN} hypothesis results ($\beta = 0.843$, t -value = 5.335, $p < 0.001$) significantly and positively showed the influence of SN on BI. This implies that opinion of specific referent groups is also important in the development of an intention towards the use of PPRSS. This result can be explained by the fact that the PPRSS have been just introduced to Monaragala, and hence the knowledge of RSs remains rather limited. As a result, they may consult people from their own social environment to seek for advice in the process of ADN of this new PPRSS. The positive relationship also denotes that, the more a person has a favorable social influence in ADN of PPRSS, that the more favorable his intention would be. The H_{PBC} hypothesis of this study shows that PBC affected on adoption of PPRSS was positively and significantly supported ($\beta = 0.583$, t -value = 3.760, $p < 0.05$). The H_{FC} hypothesized relationship i.e. that of FC and PBC too was seen to be positive and significant ($\beta = 0.645$, t -value = 4.414, $p < 0.05$). Hence, H_{FC} too was supported. The H_{BI} hypothesis of relationship of BI to ADN was significantly and positively supported too ($\beta = 0.396$, t -value = 6.091, $p < 0.001$). As effects of SN on BI towards PPRSS were comparatively higher than that of AT, policy makers should give more attention to improve the SN, which could be helped by improvement in the advisory services. Effect of PBC on ADN was seen to be comparatively higher than that of BI. This result concludes that RSs in Monaragala are

likely to engage in practicing the PPRSS, when they have the required resources to perform the behaviour. Therefore, the availability of necessary coagulation acids and skills of PPRSS should be improved to enhance adoption of PPRSS among the RSs in Monaragala.

IV. CONCLUSIONS AND RECOMMENDATIONS

The nine-predictor conceptualized model explained 77% of the variance in ADN of PPRSS by RSs in Monaragala. This study identified nine behavioural factors; PU, PEU, CO, RA, BI, AT, SN, FC and PBC that act as drivers for ADN of PPRSS. Thus, in order to enhance the ADN of PPRSS, a favorable environment contributing to these psychological factors should be improved among the RSs in Monaragala.

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EVALUATION OF *IN-VITRO* ANTHELMINTIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF BARK AND LEAVES OF *Crataeva religiosa*

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Abstract

Crataeva religiosa belongs to the family capparidacea. It exhibits a variety of pharmacological properties and therefore has been used in indigenous medicine for treatment of different ailments. Aqueous and ethanolic extracts of bark and leaves of this plant were evaluated for *in-vitro* anthelmintic activity using an adult earth worm, *Eisenia fetida*. Different concentrations (10, 20, 50, 100 mg/mL) of aqueous and ethanolic extract of *Crataeva religiosa* were used in this study. Mebendazole at the concentration of 20 mg/mL was used as a standard, and saline was used as a control. *In-vitro* anthelmintic activity was evaluated by determining the time taken to paralyze and death of *Eisenia fetida* against different concentrations of extracts, mebendazole and saline. All tests were performed in triplicate. One way ANOVA followed by Tukey's post hoc was used to compare the activities between extracts and mebendazole. According to the results, concentration-dependent activities were observed for all extracts on paralyzing or killing of

worms. The ethanol extract of leaves and aqueous extract of bark have shown higher efficiency in anthelmintic activity than their counterparts. Both leaves and bark extracts exhibited a significant difference in anthelmintic activity compared to mebendazole. We also observed that the death time of worms was lower in aqueous and ethanol extracts of bark at 100 mg/mL than in mebendazole ($p < 0.05$). In conclusion, the anthelmintic activity of the leaf and bark of *Crataeva religiosa* is observed in this study. Further studies are needed to screen the phytoconstituents of the plant that are responsible for the anthelmintic activity.

Keywords: *Crataeva religiosa*, Anthelmintic activity, Evaluation, Bark, Leaf

I. INTRODUCTION

Crataeva religiosa is a branched deciduous tree, belongs to the family capparidacea. It is distributed in the countries in tropical zone such as India and Sri Lanka. This plant is called as 'lunuwarana' in sinhala and 'mavilanku' in tamil. Root bark, stem bark and leaves of this plant

are used for various medicinal treatemnts in indigenous medicine. The different parts of this tree exhibit various pharmacological properties like diuretic, anti-inflammatory, laxative, antioxidant, antioxaluric, hepatoprotectant, lithotriptic, antirheumatic, antiperiodic, antimycotic, contraceptive, antipyretic, antilithitic, rubefacient and vasicant properties. The bark of the *Crataeva religiosa* is used in treatment of urinary disorders and kidney stone [1].

Parasitic infections affect human health causing various complications such as gastrointestinal disorders, malnutrition, anaemia, allergies and sometimes life-threatening if untreated. Although several synthetic drugs are available in treating helminth infections, discovery of new drugs needed against them due to resistance emerging against them and side effects of the synthetic drugs. Plant-based molecules have been shown promising results in the discovery of new drugs against various ailments. Even modern pharmacopoeia still consists at least of 25% of drugs derived from plants. [2] Several *in-vivo* trials have been conducted for the evaluation of the anthelmintic activity of compounds from plant origin. They showed activity by the expulsion of worms from their hosts or reducing no eggs per faeces compared to standard drugs. [3] Therefore, the present study was undertaken to evaluate *in-vitro*

anthelmintic activity of leaf and bark extracts of *Crataeva religiosa*.

II. MATERIAL AND METHODS

A laboratory-based experimental study was conducted. The aerial parts (leaf and bark) of *C. religiosa* were collected from the Jaffna district.

Collection and preparation of sample

The leaf and bark of *C. religiosa* were collected and air-dried in the shade at room temperature. Dried plant material was grounded to powder using an electric grinder. The extract was prepared with distilled water and ethanol using soxhlet apparatus.

Preparation of extracts

One hundred grams of the powdered plant material was mixed with 250 mL of solvents and extraction carried out using soxhlet apparatus for 1.5 hours. Then it was allowed to cool to room temperature. The resultant extract was concentrated using a rotary evaporator, and the extract was kept at 4 °C until further usage. The extract yield (%W/W) from plant material was calculaed [4].

Experimental worm

The adult earthworms (*Eisenia fetida*) wereas used due to its anatomical and physiological resemblance with the intestinal roundworm parasites of humans [5]. The worms were collected from the Department of Biology, Faculty of Agriculture, University of Jaffna.

They were washed initially with tap water followed by normal saline to remove the soil particles, debris and faecal matters.

In-vitro anthelmintic activity



Figure 1. Adult earthworm (*Eisenia fetida*)

The anthelmintic activity was performed on adult earthworm *E. fetida* according to the method described in previous studies [6]. This test was performed in triplicate. The worms were placed in a Petri dish containing different concentrations (10, 20, 50, 100 mg/ml) of aqueous and ethanolic extracts of *C. religiosa*.

Two worms were placed in each petri dish and observed for paralysis or death of the worms (Figure 2). The time taken for paralysis was noted when no movement was observed in the worms. It was further confirmed by shaking or giving external stimuli to the worms. Death was confirmed by loss of their motility followed by fading of their colour. Mebendazole (20 mg/ml) was used as standard drug, and saline was used as control.



Figure 2. In-vitro study of anthelmintic activity using *Eisenia fetida*

Statistical analysis

All results were presented as mean with standard deviation. One-way ANOVA followed by Tukey's post hoc was used to compare the activities between extracts and mebendazole.

III. RESULTS AND DISCUSSION

Percentage extraction yield of *C. religiosa* leaves using ethanol and water were 4.92% and 5.82% respectively. The percentage yield of *C. religiosa* bark extracted using ethanol and water were 5.27% and 4.56% respectively.

Time durations to exhibit Paralysis and death - for water and ethanol extracts of leaf and bark of *C. religiosa* at different concentrations were given in Tables 1 and 2.

According to the results, concentration-dependent activities were observed for all extracts on paralyzing or killing of worms. As shown in the Figure 3, among the leaf extract of *C. Religiosa*, aqueous extract at 100 mg/ml and ethanolic extract at 50 and 100mg/ml showed activity comparable to mebendazole. As

shown in Figure 4, among the bark extracts, aqueous and ethanolic extracts at 50 and 100 mg/ml showed comparable activity to mebendazole. The ethanol extract of leaves and aqueous extract of bark have shown more efficiency.

Leaves and bark extracts exhibited a significantly higher in anthelmintic activity compared to mebendazole. We also observed that the death time of worms was lower in aqueous and ethanol extracts of bark at 100 mg/mL than in mebendazole ($p \leq 0.05$).

Table 1. Anthelmintic activity of *Crataeva religiosa* leaf extract

Extracts	Concentration (mg/mL)	Paralyzed Time (min) Mean \pm SD	Death Time (min) Mean \pm SD
Aqueous extract	10	—	SWAM
	20	—	SWAM
	50	—	SWAM
	100	19.13 \pm 0.15	23.5 \pm 0.4
Ethanol extract	10	—	SWAM
	20	—	SWAM
	50	17.5 \pm 0.3 ^a	29.93 \pm 0.83 ^a
	100	4.33 \pm 0.15 ^b	7.2 \pm 0.3 ^b
Mebendazole 20mg/mL	20	4.53 \pm 0.5	8.47 \pm 0.5
Saline	—	—	SWAM

SWAM-survived with active movement even after one hour. Mebendazole: standard; Saline: control. Means within the column without a common superscript

letter differ at $p \leq 0.05$ compare to mebendazole.

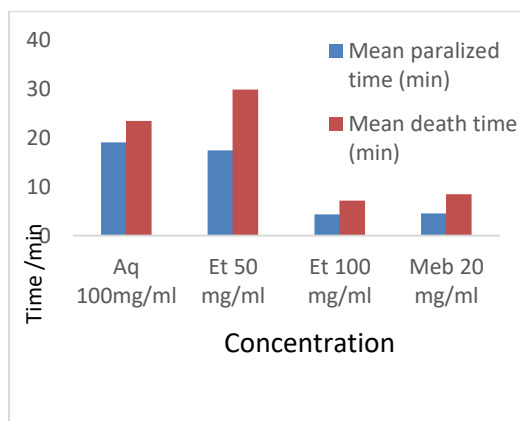


Figure 3. Mean time duration for paralyzing and death in earthworms in the presence of leaves extracts of *C. religiosa*

Table 2. Anthelmintic activity of *Crataeva religiosa* bark extract

Extracts	Concentration (mg/mL)	Paralyzed Time (min) Mean \pm SD	Death Time (min) Mean \pm SD
Aqueous extract	10	—	SWAM
	20	—	SWAM
	50	5.83 \pm 0.15 ^a	12.60 \pm 0.65 ^a
	100	1.97 \pm 0.15 ^b	3.37 \pm 0.32 ^b
Ethanol extract	10	—	SWAM
	20	80.23 \pm 1.32 ^a	172.13 \pm 2.97 ^a
	50	25.77 \pm 0.25 ^b	50 \pm 0.3 ^b
	100	4.70 \pm 0.2 ^c	5.40 \pm 0.17 ^c
Mebendazole 20 mg/mL	20	4.53 \pm 0.5	8.47 \pm 0.5
Saline	—	—	SWAM

SWAM-survived with active movement. Mebendazole: standard; Saline: control. Means within the column without a common superscript letter differ at $p \leq 0.05$ compare to mebendazole.

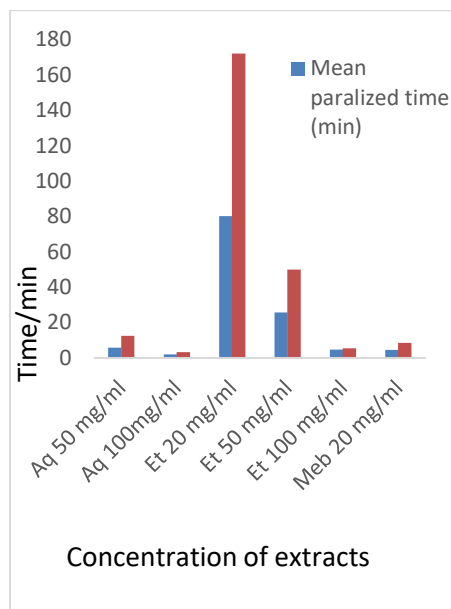


Figure 4. Mean paralyzed and death times of worms by bark extracts of *C. religiosa*

Anthelmintic activity of *C. religiosa* leaf extract

The mean time take to paralysis the worm in aqueous extract of *C. religiosa* leaf at 100 mg/mL (19.13 \pm 0.15 minutes) was significantly higher than the time take to paralysis in ethanol extract at 100 mg/mL (4.33 \pm 0.15 minutes), $p < 0.05$. However, in ethanol extract, the mean time taken to paralysis the worm at 50 mg/mL was approximately four-fold higher (17.5 \pm 0.3 minutes) than the time take to paralysis at 100 mg/mL (4.33 \pm 0.15 minutes) ($p < 0.05$). It was observed that the time taken to paralysis the worm in the aqueous extract at 100 mg/mL was slightly higher (19.13 \pm 0.15 minutes) than

the time taken to paralysis in ethanol extract at 50 mg/mL (17.5 ± 0.3), indicating the efficiency of ethanol extract on a paralysis of worm.

We also observed that the paralyzing time in mebendazole at 20 mg/mL (positive control) was 4.53 ± 0.5 minutes, which was not significantly different from the time taken to paralysis in ethanol extract at 100 mg/mL (4.33 ± 0.15) ($p > 0.05$), whereas killing time was significantly reduced compared to positive control. However, the time is taken to paralysis in ethanol at 50 mg/mL, and in the aqueous extract at 100 mg/mL was significantly higher than the positive control ($p < 0.05$).

Conversely, in aqueous extract at 10, 20, 50 mg/mL and in ethanol extract at 10, 20 mg/mL, the worms survived with active movements, similar to saline (0.9%) solution (negative control).

In the aqueous extract of *C. religiosa* leaf, the time taken to kill the worms at 100 mg/mL was increased by 22.84% compared to paralyzing time at the same concentration, whereas in ethanol extract, the time taken to kill the worms at 50, and 100 mg/mL was increased by 71.0% and 66.28% compared to paralyzing time in respective concentrations ($p < 0.05$). In positive control, the time to kill the worms at 20 mg/mL of mebendazole was increased by 87.0% compared to paralyzing time ($p < 0.05$).

The killing time in mebendazole at 20 mg/mL (positive control) was 8.47 ± 0.5 minutes, which was significantly different from the time taken to kill in ethanol extract at 100 mg/mL (7.2 ± 0.3), $p < 0.05$. The paralyzing time of the worm was not significantly different from positive control ($P > 0.05$).

However, the time is taken to paralysis in ethanol at 50 mg/mL and in aqueous extract of a leaf at 100 mg/mL was significantly higher than the positive control ($p < 0.05$).

Anthelmintic activity of Crataeva religiosa bark extract

The mean time taken to paralysis the worm in aqueous extract of *Crataeva religiosa* bark at 100 mg/mL (1.97 ± 0.15 minutes) was significantly lower than that at 50 mg/mL (5.83 ± 0.15) minutes ($p < 0.05$). A similar trend was observed in ethanol extract of bark that the paralyzing time and killing time was gradually decreased with increasing concentration of *Crataeva religiosa* bark extract (Spearman's rho correlation for paralysis and killing = -0.949 and 0.953; $p < 0.000$).

The mean time taken to paralysis the worm in aqueous extract of *Crataeva religiosa* bark at 50 and 100 mg/mL was significantly lower than the time taken to paralysis in ethanol extract at 50 and 100 mg/mL, respectively (At 50 mg/mL: 5.83 ± 0.15 vs 25.77 ± 0.25 minutes; at

100 mg/mL: 1.97 ± 0.15 vs 4.7 ± 0.2 minutes), $p < 0.05$. Similarly, the killing time was lower in aqueous extract than in ethanol extract at 50 and 100 mg/mL (at 50 mg/mL: 12.6 ± 0.65 vs 50 ± 0.3 minutes; at 100 mg/mL: 3.37 ± 0.32 vs 5.4 ± 0.17 minutes), indicating aqueous extract is better than ethanol extract from the bark. The paralyzing time taken in ethanol extract of bark at 100 mg/mL (4.7 ± 0.2 minutes) was slightly higher than in positive control ($p > 0.05$), whereas killing time was significantly lower in ethanol extract at 100 mg/mL than positive control ($p < 0.05$).

Comparison between leaf and Bark on the effectiveness

The killing time of worms at 100 mg/mL of *C. religiosa* leaf in the aqueous extract was significantly higher than that in bark extraction ($p < 0.05$). Conversely, the killing time of *Crataeva religiosa* bark in ethanol extract was significantly lower than that in bark extraction at 50 mg/mL ($p < 0.05$), whereas the killing time of *C. religiosa* bark in ethanol extract was significantly higher than that in bark extraction at 100 mg/mL ($p < 0.05$).

Anthelmintic activity and phyto constituents of C. religiosa

Several studies reported that the phytocompounds that show the anthelmintic activity include saponins, alkaloids, polyphenols,

tannins, etc. Different phytoconstituents act as anthelmintic agents in different mechanisms. *Crataeva religiosa* consists of various phytoconstituents such as alkaloids, flavonoids, glycosides, terpenoids and saponins [8]. Isolation of individual phytoconstituents and screening for activity is needed in further studies to find new chemical entities with anthelmintic activities.

Anthelmintic activity of other genus of Crataeva

Anthelmintic activity of *C. religiosa* was first time reported in this study. However other genus of *Crataeva* such as *Crataeva unilocularis* (9) and *Crataeva nurvala* (10) also showed anthelmintic activity in *in-vitro* studies.

IV. CONCLUSION AND RECOMMENDATIONS

Higher concentration of extracts of Leaf and bark of *Crataeva religiosa* showed higher anthelmintic activity compare to standard. Further studies are needed to isolate the phytoconstituents of the plant that are responsible for the anthelmintic activity and their efficacy in *in-vivo* studies.

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AN ATTEMPT TO BUILD AN EVIDENCE-BASED PLAYER RANKING SYSTEM FOR TALENT IDENTIFICATION IN SRI LANKAN FOOTBALL

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Abstract

Though being the most popular sport in the world, football in Sri Lanka has always been lagging behind cricket and other sports in popularity and funding. Most recently, Sri Lanka have been free falling down the FIFA World Rankings to 200th place out of the 211 footballing nations. The primary motivation of this study is to attempt to arrest the downfall of Sri Lankan football by introducing an evidence-based player ranking system in order to accelerate the professionalization of the sport in Sri Lanka.

For the above purpose, a collection of player performance data from 5 completed seasons of the Dialog Champions League was collated. A descriptive analysis helped identify the most important features for each playing position.

Unsupervised k-means clustering was used to group players and showed previously identified 'super players' being grouped together in the clusters discovered. Owing to the inadequacy of the data available, a richer English Premier League player performance data-set, together with FIFA ratings for each player was used to build player rank prediction models using

supervised learning in order to induce a transfer learning based predictive model for the less comprehensive Dialog Champions League player data-set. In order to be able to transfer the model in a valid way, only the features available for the Dialog Champions league were used in building these models.

The models built for defenders, midfielders and forwards showed state-of-the-art accuracies of 82%, 88% and 95% respectively, while the goalkeeper model's accuracy (71%) was slightly lower. The outcomes of this study include the ability to have a transparent selection process for the national team and a system for clubs to evaluate which players to contract, based on an evidence-based ranking system to motivate players to improve their performance – all of which would contribute to professionalizing the sport in Sri Lanka.

Keywords: Soccer analytics, Football player ranking, Sri Lankan football

I. INTRODUCTION

Football which is also called soccer, is the most popular sport in the world, played by over 265 million players in over 211 countries. The FA (Football Association of England) is the oldest

football association in the world and is regarded as the creator of the Laws of the Game in 1863. FIFA (Fédération Internationale de Football Association) is the international governing body of football founded in 1904.

In addition, each country has their own Football Association which primarily organizes a local club football league. The Football Federation of Sri Lanka (FFSL) organizes the Dialog Champions League (DCL) which was started in 1985. The DCL currently consists of 18 teams, playing in a single round-robin format. The league operates on a promotion and relegation system with the KIT Premier League Division I. Sri Lankan football has been in decline for the last 25 years with Sri Lanka falling to its worst ever position (200th out of 211 nations) in the FIFA World Rankings in November 2017, after losing in the 1st round of qualifications of the FIFA World Cup 2018 to the worst ranked team in the world at the time, Bhutan (211th). Figure 1 shows the decline of Sri Lankan football over the last 25 years.



Figure 1. Decline in Sri Lanka's FIFA ranking over time (source: fifa.com)

Despite having a club football league system, Sri Lanka has failed to

professionalize football, leading to this decline. While there are many reasons for Sri Lanka's failure in football, this study attempts to deal with improving the evaluation aspects of the game by bringing about an evidence-based approach to detecting and selecting players in the league.

The main objective of this study is to accelerate the professionalization of football in Sri Lanka by identifying the best players in the country. The method proposed to reach this objective is to identify the most important performance attributes for each playing position and to develop a player ranking system based on the most important attributes for each position.

Traditional methods of talent identification by measuring physical and psychological factors have been outdated and the current trend is to use player performance data to identify the best players. However, this comes with a new set of challenges, since there are so many factors which can affect player performance and the lack of an evidence-based method to identify the most important factors.

A previous attempt to predict player performance using data is described by Mu [1], who uses an analytical hierarchical process to challenge the official selection of Lionel Messi as the winner of the Golden Ball of the 2014 FIFA World Cup. As the FIFA Castrol Index showed that Messi was only the 5th best ranked performer at the World Cup, this ranking system tries to weigh in on the debate. Pappalardo et

al. [2] developed a system called PlayeRank, a ranking which also takes into account a player's position. This system agreed with expert opinions, validating the study further. McHale, Scarf and Folker [3] explain how the EA Sports Player Performance Index works to assign ratings to players. Its specialty is that it uses player contributions to winning performances, disregarding player positions. Kharrat, Peña and McHale [4] tried to adapt the plus-minus player rating method used in basketball and ice-hockey to football. They use this plus-minus rating to determine the best players in European football and evaluate how their ratings change with time. Matano et al. [5] combine the Adjusted Plus-Minus (APM) system for football with the FIFA rating system to build an Augmented APM player ranking. These studies show that Sri Lanka could benefit by such analytical approaches which help remove biases in selection currently plaguing much of its sports.

An evidence-based approach to player ranking has the potential to identify the most significant interventions required to arrest the downward trajectory of Sri Lankan football. The above-mentioned player ranking system will help increase the competitiveness among teams in the top league, which will in turn improve the competition for places in the Sri Lankan national team – uplifting the standard of football in Sri Lanka.

The methods used in this study can be applied across the league football system in Sri Lanka, but the scope of this research is limited to the data

available to us, as collected by the FFSL. Team and player performance are also of course affected by team management, football administration, motivation and political factors all of which are out of the scope of this study.

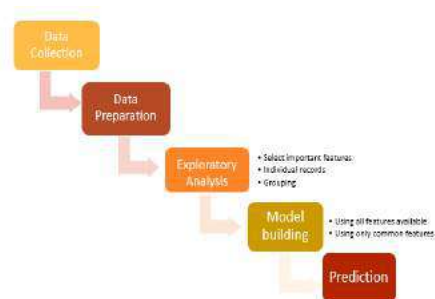


Figure 2. Main steps in the research process

II. MATERIALS AND METHODS

This study uses abductive research design on secondary data collected by the FFSL for 5 seasons of the Dialog Champions League spanning from 2012 to 2017. The 16 variables available in this dataset included, goals, assists, shots, shots on target, tackles, tackles won, fouls, yellow cards, red cards, offsides, crosses, corners, touches, passes, interceptions and blocks. The data collected by the FFSL was prepared for the purpose of this research by aggregating the data for each player separately. The players in the data-set were divided into 4 main groups based on player positions, namely, forwards, midfielders, defenders and goalkeepers. This was after Nsolo [6] who attempted to value football players in the top 5 European leagues. In his study, players were first divided into the positions of

goalkeepers, defenders, midfielders and forwards. Figure 2 shows the main steps that were followed in our research process.

The first part of the exploratory analysis consisted of identifying of the most important variables for each player position. Next, these important features were used to identify outliers using scatter plot matrices. Then, all variables were used to cluster the data by each player position separately. Clusters were evaluated using the Davies-Bouldin Index. This methodology follows Kumar [7] who first identified the most important attributes in order to predict the expert ratings of players and then predicted the match outcome using team performance data. We employed the Arndt and Brefeld [8] approach to predicting the performance of football players by proposing a multiple-regression feature elimination strategy to identify the most relevant features for predictions. We also used the Baron et al. [9] approach of identifying the most important attributes that influence a football outfield players' performance. We combine the above methods to build a model to predict Sri Lankan football players' performance levels.

Model building cannot be carried out by using only the DCL data-set as player ranking is not available in Sri Lanka to be used as a target variable. Therefore, the most significant variables for a ranking system had to be learnt from an international data-set. For this

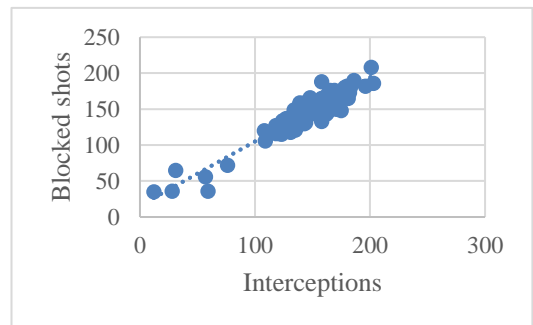


Figure 1. Blocked shots vs. Interceptions for defenders

purpose, an English Premier League (EPL) player data-set along with the FIFA rating for each player (as the target variable) was used to learn a mapping between attributes and player rating. This was inspired by Pariath et al. [10] who attempt to build a player ranking and valuation system for Indian football.

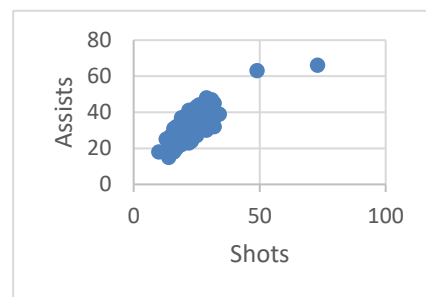


Figure 4. Assists vs. shots for midfielders

However, the models learnt from EPL data cannot be directly applied to the Dialog Champions League data, as they are based on more variables (54), many of which were not available in the DCL data. Therefore, only the variables equivalent to those available in the DCL dataset were used to build the supervised learning model with the

EPL data. The FIFA rating was used as the target variable for machine learning algorithms used for model building namely, Multiple Linear Regression, Support Vector Machines, Gradient Boosted Trees and Neural Networks. The root mean square error (RMSE) and Spearman's Rho were used to evaluate the models.

III. RESULTS AND DISCUSSION

The initial single variable exploratory analysis showed the most important variables for each player position. Variables such as goals, assists, shots, shots on target and shot accuracy were seen to be important for ranking forwards when compared with other player positions. Similarly, tackles, tackle success, fouls, interceptions and blocked shots were identified as important variables for ranking defenders. It was also seen that midfielders needed all the variables in moderation, while their rankings showed slightly more importance for passes and touches than other player types.

Some unexpected outcomes were generated in terms of potential sub-player categories being identified in the midfielder player comparisons. Attacking midfielder players, defensive midfielder players and all-round midfielder players were found to have distinguishable key attributes. Among the defenders, Mohammed, Jayashantha, Gunathilake and Priyadarshana were highlighted as players with strong defensive attributes, while Rizvi, Rishad, Ruwan and Ayurdeen were identified as the better goalkeepers in the dataset.

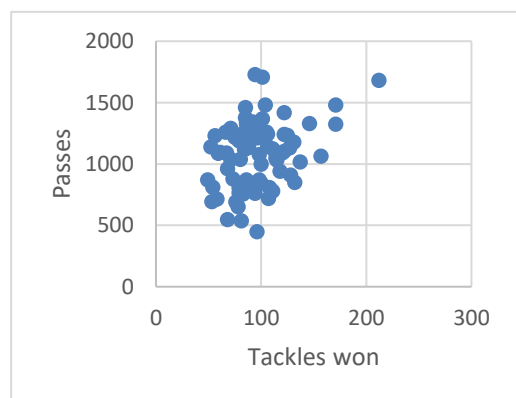


Figure 5. Passes vs. tackles won for midfielders

Based on the results of the preliminary identification of important variables, scatter plots were generated for each player position using these identified variables. When observing the defender scatter plots, six below average defenders were identified as outliers as shown in figure 3. Contrastingly, the midfielder scatter plots (figures 4 and 5) in the scatterplot matrix, show three above average performers as outliers.

As the next step, unsupervised k-means clustering using all 16 variables was performed for each player position separately. The clustering of the forwards as expected, resulted in most of the highly skilled forwards identified in cluster 2 (shown in Table 1).

Table 1. Forwards clustering

	Goals	Goals per match	Touches
Cluster 0	30.96%	9.44%	58.36%
Cluster 1	7.07%	32.61%	94.11%
Cluster 2	18.76%	14.99%	13.81%
on average % lower			
on average % higher			

The findings of the sub-player categories for midfielders was further strengthened as the attacking midfield players identified by the player comparison analysis were also grouped together in the clustering. In the defender clustering, one cluster appeared to contain the best attacking full backs in the game. Two defenders provided a high number of crosses and assists when compared to other defenders. The defenders with the high defensive attributes as identified in the player comparison analysis, were grouped together in clustering as well. Finally, 4 defenders present as outliers in the defender's scatter plot were grouped together. In clustering of goalkeepers, Ruwan, Rishad, Ayurdeen and Rizvi were grouped together in cluster 1 (shown in Table 2) as the best goalkeepers in the league, strengthening the findings in the player comparison analysis.

Table 2. Goalkeeper clustering

	Clean sheets	Saves	Shots faced
Cluster 0	76.77%	72.01%	70.72%
Cluster 1	118.65%	111.28%	109.3%
on average % lower			
on average % higher			

As a final step, supervised model building was performed using only the 16 variables available in the DCL on an English Premier League dataset.

Feature selection was performed initially, using the significant coefficients of the Linear Regression models, resulting in the following variables for the deferent player position models: for defenders wins, passes per match, blocked shots, losses, crosses and red cards; for midfielders wins, shots on target, passes per match, fouls, interceptions, losses, passes and yellow cards; for forwards appearances, goals per match, assists, passes per match, losses, shooting accuracy and passes and for goalkeepers saves, losses and clean sheets.

The target predicted was the rating of the individual players, which was compared to the official FIFA rating. This comparison is most meaningfully performed by computing the Spearman's Rho of the resulting ranking of the predicted with the ground truth rating based ranking. This Rho value is reported below as the model accuracy. Four machine learning algorithms were used to build models for each player position separately and all performed above or near the 80% score on accuracy as seen in Table 3.

Table 3. Model accuracies by Machine Learning algorithm

ML Algorithm	Accuracy
Linear regression	84%
Support Vector Machines	87%
Gradient Boosted Trees	79%
Neural Networks	77%

Three of the four player position models fitted very well with only the goalkeeper model needing improvement. This can be attributed to the fact that the number of variables collected for goalkeepers in the DCL being low (4). Table 4 shows the accuracies of the models classified by player position.

Table 4. Average accuracy by player position

Player position	Accuracy
Forwards	95%
Midfielders	83%
Defenders	78%
Goalkeepers	62%

The rankings shown in Tables 5, 6 and 7 were obtained when the models trained on the restricted EPL data were applied to the Dialog Champions League dataset. Only players present in all 5 seasons of the data available were used to build this player ranking owing to the need for sufficient data for the model building process.

Table 5. Top 5 forwards - Neural Networks

Rank	Name
1	Bashith Ameer
2	Gonalagoda Pedige
3	Gerrard Perera
4	Menaka Jeran
5	Suresh Sanjeev

Table 6. Top 5 midfielders - Neural Networks

Rank	Name
1	Shanushka Viduranga
2	Danushka Madushanka
3	Tharaka Damith
4	Hanifa Mohamed Fazil
5	Chathuranga Sanjeewa

Table 7. Top 5 goalkeepers - Neural Networks

Rank	Name
1	Mohamed Rizvi
2	Fazeel Ayurdeen
3	Kaveesh Fernando
4	Ajith Kumara
5	Nazeer Imran

The majority of the top 5 players in each position are players seen in previous steps in the analysis, validating the results of the supervised models built.

IV. CONCLUSIONS AND RECOMMENDATIONS

The best accuracies for player ranking models for forwards of 95.3% (Gradient Boosted Trees), for midfielders of 88.8% (Support Vector Machines) and for defenders of 82.4% (Linear

Regression), are all at state-of-the-art levels. The model built for goalkeepers has a relatively lower accuracy (71%) mostly due to the limited number of variables collected for goalkeepers. This could be resolved with minimal additional effort and cost by collecting just 2 additional variables for goalkeepers (passes and sweeper clearances) which would potentially increase the accuracy up to nearly 78%.

The player ranking models built in this study can be used to rank players at the end of the ongoing season. It has many uses, some for football clubs, others for the football administration and yet others for individual players.

Football clubs can also use it to compare players in order to select which player(s) to contract for an upcoming season. Football administrators such as the FFSL could use the ranking system to evaluate the most valuable player(s) for national pool selection, and for the award of accolades at the end of each season. A player valuation system or even a pre-season and mid-season auction could be also be held using the proposed ranking system.

Furthermore, if this ranking system is adopted by the FFSL to rank players throughout the season, it would motivate players to improve their performances to progress up the ranking.

Finally, if the FFSL uses this evidence-based and transparent ranking system

to select players for the national team, it would remove the biases of the selectors. This would raise the standard of football and lead to increased competition for places in the national team – leading to the development and professionalization of football in Sri Lanka.

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A STUDY OF THE USE OF METACOGNITIVE STRATEGIES IN THE TEACHING LEARNING PROCESS OF MATHEMATICS

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Abstract

The psychological concept of metacognition refers to engaging in work with knowledge and focusing on oneself and the work and monitoring it with a conscious mind. Metacognition helps the teacher control his or her behavior in the learning-teaching process and makes a difference in the student's behavior. This provides an opportunity for the students to further enhance their learning. The objectives of the research are to identify the metacognitive strategies that can be used in the teaching of mathematics to influence the subject matter and to examine the cognitive strategies of the mathematics teachers. To identify the impact of metacognitive strategies on subject performance, according to the investigative method, eighty-four students were selected from two parallel classes in grade eleven to teach using traditional methods and metacognitive strategies. For pre-test and post-test, a question paper based on geometry was used. To investigate teacher Cognition on metacognitive strategies, a questionnaire prepared with the help of the metacognitive transcript adopted by Schraw and

Dennison in 1994 was modified and used to suit the Sri Lankan teaching community. The sample was taken from all the math teachers in 1 AB, 1C, and type two schools in the Nivithigala Education Zone in the Ratnapura District. Analysis of data from the Evidence mediation also revealed that teachers do not have a high level of awareness of metacognitive strategies when teaching mathematics. Accordingly, awareness should be given to teaching geometry through the use of metacognitive strategies, and opportunities should be provided to enhance the teaching ability through the use of metacognitive strategies for the development of the teaching profession.

Keywords: Mathematics, Teaching-learning process, Metacognitive strategies

I. INTRODUCTION

When analyzing any daily task that we do daily, it is clear that mathematical concepts are used to facilitate many activities we do in human society. Mathematical concepts are therefore essential to human life. Moreover, mathematical ability is a factor that

directly affects the economic, social, and spiritual development of the country. Furthermore, mathematics is a subject that helps in the development of high mental ability. Therefore, Mathematics in Sri Lankan schools has been a subject taught in classrooms for thirteen years from Grade 1 to Grade 13 and it has been made a compulsory subject for all students up to grade eleven.

Against this background, the percentage of students who have passed the GCE Ordinary Level Mathematics in Sri Lanka is an average of 57% [6, 7, 8, 9, 10]. Thus, an average of 43 percent fail mathematics each year. Moreover, students who fail to get an average pass for mathematics in the GCE Ordinary Level Examination, have to leave the school system without the opportunity to enter any stream of higher education [14]. Due to this, it has become an urgent need to monitor the teaching and learning process to improve the achievement level of Mathematics in the GCE Ordinary Level examination.

The GCE Ordinary Level Mathematics Question Paper is structured to measure the content of six subject areas including Numbers, Measurements, Algebra, Sets, Probability Statistics, and geometry. The percentage of marks covered by each theme in the question paper is 23, 15, 20, 10, 10 and 22 respectively [15]. The fact that the coverage percentage

of marks is 22 indicates that geometry is one of the most important themes in the overall subject. Nevertheless, the number of students who choose the twelfth question which is to be answered with the knowledge of geometry in the second part of the GCE Ordinary Level Mathematics question paper is very limited. (Table 0)

Table 1. The percentage of students who have selected the twelfth question related to Geometry in part II of the GCE Ordinary Level mathematics question paper

Question No	Subject Theme	2010	2011	2012	2013	2014	2015
12	Geometry	36%	55%	35.4%	25%	26%	26%

Source: GCE Ordinary Level Examination (2010-2015) assessment reports

Even the students who chose the twelfth question got very low marks for that question (Table 2).

Table 2. The mediation of marks obtained for the twelfth question related to the geometry of part II of the GCE Ordinary Level mathematics question paper from 2010 to 2015

Year	Marks between 0-2	Marks between 3-5	Marks between 6-7	Marks between 8-10
2010 - 2015	65%	20%	08%	07%

Source: GCE Ordinary Level Examination (2011-2015) assessment reports

Evidence has been found in the foreign research literature that Metacognition can be used to enhance the performance in the teaching-learning process of various disciplines, including the geometry of mathematics. [16] showed that students with disabilities similar to normal students are generally more successful at solving mathematical problems when using Metacognitive strategies. Furthermore, he believed that Metacognitive strategies influence the ability of the students to solve mathematical problems. [13] points out that combining the educational process with cognitive strategies in solving mathematical problems makes a significant contribution to learning. In addition, [17] presented that students with better self-regulation and Metacognitive strategies achieve higher academic achievement regardless of the grade and the level in which they are in. Based on this foreign literature, it is clear that the use of cognitive strategies in the teaching-learning process is appropriate to enhance mathematical performance,

which is a compulsory subject in Sri Lanka.

Since no one so far was able to examine how Metacognitive strategies can be used in the process of teaching and learning mathematics which is a compulsory subject in the secondary education curriculum of Sri Lanka, the purpose of the present research is to fill that gap. In addition, the specific research objectives include identifying the effect of Metacognitive strategies on performance, which can be used in the process of teaching geometric concepts in mathematics, and investigating the cognition of the math teachers in utilizing Metacognitive strategies.

II. MATERIALS AND METHODS

To achieve the objective of understanding to what extent the cognitive strategies that can be used in the application of the geometric concept of mathematics affect our performance, semi-test layouts with

unequal groups of pre-and post-test content were used. Pre- and post-test group samples include randomly selected 84 students (the experimental group included 42 students and the control group included another 42 students) from two parallel classes of grade 11 in a particular college in the Ratnapura district.

For the students of the experimental group, teaching was done with the use of Metacognitive strategies while for students in the control group the teaching was done with the use of traditional methods. Data were collected from tests performed before and after this intervention.

A. How the two classes were taught geometric concepts using Metacognitive methods and traditional methods

Because students in grade eleven were used in the probationary test and as by that time, the third term math lessons had to be taught, lessons on the subject of geometry were selected from the lessons of that term. As twenty-nine periods have been allocated for the third term geometry lessons of the eleventh-grade mathematics subject, twenty-nine lesson plans were developed for the students in the experimental group using the Metacognitive strategies to cover all of those lessons, and the students in the control group were taught using traditional methods. Among the strategies of Metacognition, planning strategies, thinking out loud, generating problems, and the

strategies of problem-solving were used in the preparation of lesson plans. Below is a description of how each one of them used the strategy.

According to [2], to determine learning activities and planning objectives, with the raising of a question or a set of questions, the strategy is the plan to develop the skill of planning the learning process according to the specific steps that begin and according to [11], it is difficult for students to autofocus when the planning and learning are done by someone else. In teaching the students of the experimental group, the lesson plans were made as the solving steps of the sum have to be planned by oneself.

When considering the strategies of generating problems, [11] showed that, questioning information is an important part of intelligence and that students should ask questions from themselves before and during the reading of the material. Furthermore, [3] showed that asking self-questions to know about oneself is an essential cognitive strategy. Accordingly, the student can maintain Metacognition by raising questions from different perspectives. It can be revealed that it is important for the teacher to raise questions about the learning process of the student first and then the students may self-question their activities to make a successful learning process.

[11] points out about problem-solving strategies as, instead of teaching the student how to solve problems, the teacher should help the student learn how to execute the order of action to solve the problem and that small groups can be used as a successful strategy to empower the ability of problem-solving in mathematics at a higher level. Furthermore, [17] pointed out that cognitive knowledge and skills usually develop slowly and independently with age, and therefore, the teachers should work to provide students with greater Metacognitive knowledge and skills. Even though the situation is so, waiting for the Metacognitive knowledge and skills to develop naturally is a waste of time. Advice is more effective when developing Metacognitive skills compared to maturity. This means that there is a responsibility for the teachers to make the learning environment for students to be organized in such a way to help them develop their Metacognitive knowledge and skills. However, to plan such lessons, teachers must first have Metacognitive knowledge and skills and they need to receive Metacognitive experiences. Here, teachers first need to educate and advise students on how to approach the problem. In other words, teachers should work to awaken the Metacognition of students.

[11] describing thinking aloud showed that talking about thinking allows those learners to identify their thinking

abilities. Also, a learner describes his thought process and further points out that his thought process can be clearly explained by directing and asking questions from his or her partner. Thus, thinking aloud can be considered as a Metacognitive exercise that is at a very high level. It includes activities such as the conversion of thoughts to vomiting, talking out loud the steps that one uses to solve their problem and saying everything out loud what they think about the problem and it is an advantage to be able to see and correct a mistake as soon as it has occurred.

Taking all these factors into consideration, the researcher set out the activities to solve the problems of geometry by herself, and the students were seen engaging in activities with enthusiasm.

B. How the students in the control group were taught using the traditional methods

In a traditional classroom, learning is often done using a blackboard to teach children what the teacher knows. In teaching the students of the control group, the lesson began with a brief reminder of past lessons and then by solving and demonstrating an example on the blackboard. The students looked at the blackboard and noted it down and to be fair for the students, they were questioned if any subject matters were difficult to understand, and in such cases, the subject matters were repeatedly explained to them. However, the students in this case did not have an opportunity to explore and

discover based on the activities. Here too all the subjects were taught by the teacher himself under the traditional method.

C. Investigating the cognition of mathematics teachers in utilizing cognitive strategies

Metacognition can be classified into two main dimensions knowledge of cognition and regulation of cognition. According to [12], the knowledge of cognition consists of three aspects such as declarative knowledge, procedural knowledge, and conditional knowledge, and the regulation of cognition consists of three characteristics such as planning, monitoring, and evaluation. After considering all these factors, the questionnaire which was passed in English by Schraw and Dennison was modified to suit Sri Lanka. It was translated into Sinhala to achieve the objective of investigating the cognition of teachers Metacognitive strategies. To achieve the objective of investigating the cognition of the

teachers on Metacognitive Strategies, this questionnaire was distributed among 110 teachers.

III. RESULTS AND DISCUSSION

A. The impact of metacognitive strategies on mathematical performance

To examine the impact of Metacognitive strategies on math performance, the pre and post-test results obtained by both the experimental group and the control group were analyzed separately with the help of paired sample test and according to the final t value ($p = 0.05$) obtained, it was clear that both groups showed significant improvement in the other test scores compared to the previous test scores (Table 3).

Table 3. Paired sample t-tests for pre and post-tests

Group	Paired Differences				t Value	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
Paired 01 Experimental Group Pre Test and Posted Test Marks	64.80	15.31	2.36	69.58 60.03	27.42	41	.000
Paired 02 Control Group Pre Test Post-test Marks	38.09	18.31	2.82	43.80 32.38	13.48	41	.000

According to Table 3 above, the average mean difference in the scores of the experimental group is 64.8095 and the average mean difference in the control group is 38.0952. These results show that the average gain difference of the group that taught mathematics using Metacognitive strategies was higher than that of the group that traditionally taught mathematics.

In addition, the paired sample t-test was also used to determine if there was a difference between the mean value of the pre and post-test results of both groups. The following are the hypothesis made based on the analysis of the test results of the two experimental and control groups;

H 0 - There was no change in the mean value of the test results before intervention

H 1 - There is a difference in the mean value of the test results before intervention

Based on the above hypotheses, when testing whether there is a difference between the previous test results of the experimental group and the control group, according to the $p = 0.05$ level, its t value was 1.097, and thus, its Sig. (2-tailed) is 0.279 (Table 04). Accordingly, there is no difference between the results of the students in the experimental group and the control group. In other words, hypothesis H 0 is accepted. Accordingly, the test results show that the student performance of the two groups was similar before teaching using Metacognitive strategies and traditional methods.

Table 4. The t-test results of the paired sample tests before intervention

Group	Paired Differences				t Value	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
Paired 03 Experimental Group and the Control Group Pre Test Marks	.73	4.36	.67	.62 2.09	1.09	41	.27

The following is the hypothesis that was made in the post-test score analysis of both the experimental and the control groups after the intervention;

H 0 - There is no difference in the mean value of the post-test scores because they were taught using Metacognitive strategies.

H 1 - There is a difference in the mean value of the post-test scores because they were taught using Metacognitive strategies.

Based on the above hypotheses, when testing whether there is a difference between the previous test results of the experimental group and the control

group, according to $p = 0.05$ level, its t value was 6.110, and thus, its Sig. (2-tailed) is 0.000 (Table 05). In other words, hypothesis H 0 can be rejected. Accordingly, there is a difference in the Sig. (2-tailed) between the scores of the students in the research group and the control group after the intervention. Accordingly, it was revealed that in comparison to teaching students using traditional methods, their achievement can be enhanced to a higher level by teaching them using Metacognitive strategies.

Table 5. The t results of the paired sample tests after intervention

Group	Paired Differences				t Value	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
Paired 04 Experimental Group and the Control Group Post-test Marks	27.45	29.11	4.49	18.37 36.52	6.11	41	.00

It is also clear from chart 01 that the difference in the median value of scores of the experimental group is significantly greater than the median value of the scores of the control group.

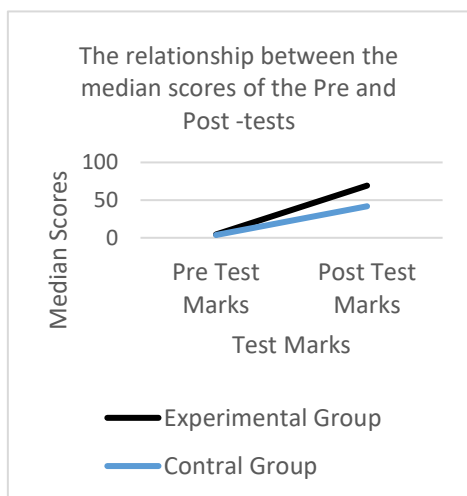


Figure 1. The relationship between the median scores of the pre and post-tests

B. The teacher cognition on metacognitive strategies

Considering the different dimensions of Metacognition, the awareness of the math teachers on Metacognitive strategies, and to explore the extent to which Metacognitive strategies can be

used in teaching, a questionnaire was designed with the help of the Meta Cognition Awareness Inventory. The patterns and the sub-patterns which are emphasized in the questionnaire are demonstrated in figure 1. In analyzing the data collected from the structured questions thus tailored to the Likert's scale, according to the response of mathematics teachers to questions used to identify different levels of cognitive strategies used in the teaching-learning process, marks were awarded. Then, a large group of variables is divided into a few sets of variables or several sets of factors, and their scores are calculated separately to analyze the paradigm shift between Metacognitive knowledge and Metacognitive regulation. To do this, the mean values of the scores were considered, and accordingly, conclusions were reached on the strategies most commonly used based on the statements which received the most responses.

C. Metacognitive knowledge

Metacognitive Knowledge was analyzed under three sections declarative knowledge, procedural knowledge, and conditional knowledge.

Declarative Knowledge

Declarative knowledge is an assessment one has about the existing knowledge of oneself. According to the data collected to the responses of the math teachers to the declarative knowledge measured from the items included in this index, from the question elements that illustrate the declarative knowledge, the question, "I know what I am expected to teach?" has received a high number of responses and its median value is 3.92. Thus, it was revealed that, in the application of declarative knowledge, the teacher acts with an awareness of what he or she intends to teach.

Table 6. Declarative knowledge and the responses of teachers

Item Number	Item	Composite Mean
1	I have an understanding of the strengths and weaknesses of my teaching	3.64
7	I am aware of what skills are most important to be a good teacher	3.42
13	I have control over how well I teach	3.66
19	I know what I am expected to teach	3.92

Procedural Knowledge

Procedural Knowledge is the knowledge that one has about his or her ability to use or teach students. According to the analyzed data, from the elements that represent procedural knowledge, the question, "I try to use teaching techniques that worked in the past", has received a high number of responses and its median value is 3.61. This means that in the application of procedural knowledge, teachers handle teaching with an awareness of how and when to use their knowledge and strategies in the teaching process.

Table 7. Procedural knowledge and the responses of teachers

Item Number	Item	Composite Mean
2	I try to use teaching techniques that worked in the past	3.61
8	I have a specific reason for choosing each teaching technique I use in the class	3.46
14	I am aware of what teaching techniques I use while I am teaching	3.59
20	I use helpful teaching techniques automatically	3.48

Conditional Knowledge

Awareness of one's knowledge and resources and environmental conditions required to use that knowledge comes under conditional knowledge. From the factors that represent conditional knowledge, the question, "I use my knowledge to compensate for my weaknesses in my teaching", has received a high number of responses and its median value is 3.23. Accordingly, it was revealed that the knowledge one possesses and the awareness which is required when using the resources, one uses his or her energy to overcome the weakness one possesses.

Table 8. Conditional knowledge and the responses of teachers

Item Number	Item	Composite Mean
3	I use my strengths to compensate for my weaknesses in my teaching	3.23
9	I can motivate myself to teach when I need to teach	3.68
15	I use different teaching techniques depending on the situation	3.66
21	I know when each teaching technique I use will be most effective	3.39

her mind for learning and teaching. What is meant by this component is, being mindful in activating knowledge or inactivation of knowledge without limiting only to awareness as it was meant in the first component.

To do this, it is important to engage in planning one own work oneself and it is also important to engage in monitoring to regulate and execute what is planned. Thus, only the teachers with Metacognitive strategies would be mindful in engaging in planning and regulating work.

Planning

Planning for teaching, going through a vision, and providing resources for it are the parts of planning. Planning is very important when regulating cognition. From the factors related to planning in the regulation of cognition, the question, "I pace myself while I am teaching to have enough time" has received a high number of responses and its median value is 3.83. It is a statement that is highly valued in comparison to other statements. This shows that a teacher is more concerned with time management when teaching.

D. Metacognitive Regulation

Metacognitive regulation is when a teacher can control his or her teaching by using a set of activities that he or she chooses to teach, in which he or she controls and directs or regulate his or

Table 9. Planning and the responses of teachers

Item Number	Item	Composite Mean
4	I pace myself while I am teaching to have enough time	3.83
10	I set my specific teaching goals before I start teaching	3.45
16	I ask myself questions about teaching materials I am going to use	3.43
22	I organize my time to best accomplish my teaching goals	3.65

2) Monitoring

Monitoring refers to understanding the ability to assess teaching or teaching strategies utilized by oneself. From the factors related to understanding the regulation of cognition, the question, “I ask myself periodically if I meet my teaching goals while I am teaching” has received a high number of responses and its median value is 3.93. Accordingly, it was revealed that teachers work with an understanding of whether they are reaching the objectives of teaching while teaching.

Table 10. Monitoring and the responses of teachers

Item Number	Item	Composite Mean
5	I ask myself periodically if I meet my teaching goals while I am teaching	3.93
11	I find myself assessing how useful my teaching techniques are while I am teaching	3.61
17	I check regularly to what extent my students comprehend the topic while I am teaching	3.66
23	I ask myself questions about how well I am doing while I am teaching	3.89

3)Evaluating

Evaluating refers to the testing of whether what is being implemented at the end of the teaching unit is successful (Analysis of performance and strategies). From factors related to evaluation in cognitive regulation, the question, “After teaching a point, I ask myself if I would teach it more effectively next time” has received a high number of responses and its median value is 3.96. It shows that after teaching a subject, one makes a self-assessment of it and thinks for oneself how to teach it better in a later situation.

Table 11. Evaluating and the responses of teachers

Item Number	Item	Composite Mean
6	I ask myself how well I have accomplished my teaching goals once I am finished	3.5
12	I ask myself if I could have used different techniques after each teaching experience	3.61
18	After teaching a point, I ask myself if I would teach it more effectively next time	3.96
24	I ask myself if I have considered all possible techniques after teaching a point	3.39

E. Findings of the Research

Depending on the nature of the pre and post-test scores, in the process of teaching mathematics, it is apparent that the use of Metacognitive strategies can improve the performance.

According to the responses received for the questionnaire made based on the Metacognitive Awareness Inventory, when activating the knowledge of teachers on cognition or when the knowledge is inactivation, when planning one work and in regulating and implementing what is planned and also in analyzing the performance and strategies, it was revealed that there was no high level of awareness.

IV.CONCLUSIONS AND RECOMMENDATIONS

Teaching using Metacognitive strategies can enhance mathematical achievement. Furthermore, in the process of teaching and learning mathematics, more attention needs to be paid to planning strategies, thinking out loud, problem-solving strategies, and generating questions. Since teachers need to have a clear understanding of cognition to improve the cognitive ability of the students, teachers' knowledge of cognition and regulation of cognition should be enhanced.

The following are some of the suggestions that can be implemented to be successful in the use of Metacognitive strategies in the process of teaching and learning;

By designing activities using Metacognitive strategies to teach geometry, it is important to remove the misconceptions among students about this theme to improve their achievement levels.

Inclusion of a guide into the syllabus on teaching using Metacognitive strategies in the mathematics syllabus of both junior and secondary grades.

Students of all levels including primary, secondary and tertiary, should be taught through the use of Metacognitive strategies and they

should be properly guided to achieve successful results.

Switching from traditional teaching methods to an interactive approach based on the real involvement of students in the process of teaching and learning.

It is very important to increase the awareness of teachers on Metacognitive strategies for their professional development.

The teacher trainees should be made aware of the Metacognitive strategies in their pre-service teacher training sessions.

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FOCUS AREA
Environment

AN EXPLORATION OF MAJOR THEMES OF CORPORATE SUSTAINABILITY DISCLOSURES: A QUALITATIVE STUDY USING SRI LANKAN LISTED FIRMS

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Abstract

Corporate Sustainability Disclosure is a rising trend in the modern business world as a reporting mechanism to enhance the firm's reputation and transparency and capture strategic business opportunities globally. Even though there are established frameworks for sustainability reporting, the nature and content of the reporting in different economies and country settings may differ with various external and internal institutional environmental factors. The objective of the study was to explore the major themes or content reported in corporate sustainability disclosures in Sri Lanka. The annual reports of 25 firms with top market capitalization were selected from the Colombo Stock Exchange firms, and a thematic content analysis was conducted. For the analysis, the Sustainable Development Goals (SDGs) was chosen as the basis as a renowned set of guiding principle for the nations to achieve sustainable development. The

results reveal that 17 sustainability-related information were found in Sri Lankan corporate Sustainability reports. The most frequently reported content in Sri Lankan Sustainability reports was human and societal related content, followed by economic and environmental aspect reporting. The firms in Sri Lanka have a trend in reporting on their contribution to generations of economic capital within the firm and an exceptionally lower tendency in reporting on their contribution to protecting the beaches and water capture areas. The results lead to significant findings on aspects the firms have reported on their sustainability performance in Sri Lanka, disregarding the difference in guidelines and frameworks used. As Sri Lanka is a nation progressing towards sustainable development with the collaboration of the United Nations, the results would be significant for the industry policymakers in designing a common of guidelines that would best suit the economy of Sri Lanka and

towards sustainable development of the country.

I. INTRODUCTION

The operating context of the businesses has been changed dramatically over the past few years with the damages to nature, climatic changes, working conditions over human rights issues and social unrest [1]. Moreover, the global pandemic situation has aggravated the unequal distribution of economic opportunities over the world. Underlying numerous problems, the inclination of the firms towards sustainable practices is conceivably a growing trend as it uncovers innumerable options to the business communities amidst the prevalent turbulent environment.

The term “Sustainability” was introduced from the report “Our Common Future” by the Brundtland commission in 1987. Sustainability was then referred to as the development that encounters the needs of the present generation without compromising the ability of the future generation to meet their own needs [2]. The latest concept of sustainability is grounded on integrating the three pillars of sustainability or three non-substitutable types of capital; economic, social, environmental or natural capital.

Keywords: Corporate sustainability reports, SDGs, Thematic content analysis

In 2015, the United Nations formulated the Sustainable Development Goals (SDG) as an urgent call to achieve a better and sustainable future for all. Reporting on the sustainability performance was considered a method of disclosing firms’ strategies towards a sustainable future and communicating their legitimacy to their stakeholders. SDGs offer a foundation for sustainability reporting practices and guide the firms to achieve a sustainable future. Nevertheless, the lack of a consistent reporting framework comparable to the formulated SDGs persisted in being an unresolved problem [1]

The GRI guidelines are the most extensively identified framework and the dominant global standard practice for corporate sustainability reporting [3]. However, there are variations in the understanding and adaptation of the guidelines by the business firms. Extensive work has been conducted by the United Nations Global Compact (UNGC) and the Global Reporting Initiative (GRI) to identify the key actions and disclosure themes for sustainable performance and reporting based on the SDGs [4] as it is a much-acknowledged set of goals throughout the world. However, a standard set of guidelines is still under consideration to have a more

standardized framework that every firm could practice equally to encourage comparability of data between firms [1, 4]. In the absence of a uniform framework for sustainability reporting, uneven reporting content could presumably exist among the disclosures in different nations.

Asia Pacific region has shown to have a higher growth rate of eighty-four per cent (84%), second to the American region with the highest Sustainability reporting rate. A Lower-middle-income developing country like Sri Lanka has sustainability reporting rates lower than the global average rate [3]. Similar to many other developing countries, sustainability reporting in Sri Lanka is not mandatory for business firms according to the laws and regulations of the country.

This research aimed to examine the sustainability reporting practices of Sri Lankan firms by identifying the commonly reported themes in their corporate sustainability reports. The previous research on the sustainability reporting practices of Sri Lanka is minimal. Several studies have explored the determinants and impacts of corporate sustainability reporting [5, 6].

While several qualitative studies have been conducted previously by employing content analysis based on the GRI guidelines, none of these studies refers to the SDGs as a foundation in identifying the significant themes reported in

corporate sustainability disclosures. Thus, this study uses the thematic analysis technique to determine the commonly reported themes based on SDGs in the sustainability reporting by listed firms in Sri Lanka.

The rest of the paper is organized as follows. Section 2 will be an interpretation of the methodology adopted for the qualitative analysis. Section 3 would be presenting the results and discussion of the thematic analysis. Finally, section 4 will give a conclusion on the overall findings with showing the policy implications.

II. MATERIALS AND METHODS

Thematic content analysis, which is a qualitative analysis technique, was employed for the analysis. A sample of 25 corporate annual reports in the year 2019 has been selected for the study. The companies were selected based on the highest market capitalization reported on June 2020 in the Colombo Stock Exchange (CSE). Previous researchers have identified that market capitalization: the measurement of the firm's size is a significant factor that positively influences the sustainability reporting of the business firms [7, 8, 9]. Thus, the firms with the highest market capitalization were selected for this study, aiming to obtain a rich extract of sustainability reporting contents.

Thematic content analysis is a well-established research technique to identify, analyze and report patterns

across qualitative data. This technique is used to obtain a detailed view of the data, which cannot be determined using quantitative research methods. The NVivo qualitative analysis software was used for conducting the thematic analysis.

First, free codes or sub-nodes were identified initially while reading through the text of 3 annual reports. To increase the validity of the analysis and reduce any bias of reading, two researchers (NS and AP) reviewed the codes and themes. The coding of the qualitative data was conducted referring to the SDGs. Coding the data refers to encoding the data after reading and identifying important moments and constructing themes using the raw data. Codes refer to the labels assigned a symbolic meaning to the information compiled during the study [10]. The words, phrases denoting important sustainability aspects in the sustainability reports were coded with referring to the SDGs. Subsequently, the sub-nodes or the reporting categories were classified into focused nodes referring to the goals of the SDGs. Based on prior literature, the focused nodes were finally categorized into major themes grounded on the three pillars of sustainability [11]. Finally, this study identified the sub-nodes and nodes representing the major reported content in the corporate sustainability reports incorporating SDGs.

III. RESULTS AND DISCUSSION

GRI has allegedly provided an extensive framework for the corporate sustainability reporting of most of the Sri Lankan firms. Nevertheless, the diffusion of GRI guidelines to the Sri Lankan corporate Sustainability reporting practices may vary upon the knowledge and understanding of the specific firms' employees and the relevance of the particular GRI performance indicators on firm business operations [12]. Even though the GRI guidelines have provided detailed Key performance indicators to report on firm sustainable performance, all the performance indicators may not be reported by firms, creating a divergence in the reporting content of disclosures. Moreover, some firms appeared to use their knowledge and experience on sustainability reporting rather than following the GRI guidelines. Hence, it was intended to identify the significant reported themes in sustainability disclosures.

The summary of the thematic content analysis is shown in Table 1. 17 nodes or reporting information were identified by exploring the text in the sustainability reports. These 17 nodes reflect the key contents of sustainability disclosures by Sri Lanka listed firms. The total number of references identified in the analysis was 552.

Several key themes were identified while exploring the reported

content of sustainability reports of Sri Lanka. The conclusive themes were economic reporting content, social reporting content and environmental reporting content. However, the research objective was to identify the extent of reporting on the three pillars of sustainability and identify the significant reporting themes. The nodes under each theme better understand the reporting content in corporate sustainability disclosures of Sri Lankan firms.

Theme 1: Human and societal aspect reporting

The theme identified with the highest number of references in Sri Lankan sustainability reports was Human and Societal aspect reporting. Social sustainability could be recognized as a value addition process for communities by enhancing the human and societal capital of the communities [11]. It was acknowledged that human capital concerns the aspects of skills, inspiration and devotion of the employees and other stakeholders of the firm. In contrast, societal capital concerns quality public services such as educational systems, cultural support, entrepreneurship and infrastructure. In the study, it was identified that firms had included the information on their (1) commitment to uplifting the living conditions of the rural communities, (2) support on sustainable agricultural practices, (3) providing

health and safety facilities, (4) providing educational facilities for employees and community, (5) minimizing gender inequality and (6) ensuring ethical work practices within the workplace in their sustainability disclosures.

The focused node on firms' commitment to fulfilling timely educational needs of the community and employees was identified to be reported more frequently. This practice shows firms' commitment to improving the education of the community and employees. Investing in community based educational programs and scholarships were shown to have reported more significantly in corporate sustainability reports of Sri Lanka considering the sub-node classification. It could be observed that, based on sustainability reports, programs organized by the firms on providing scholarships and donations to school children were reported more frequently in firms in their sustainability reports. These results were well explained through the prior literature, where the author has stated that poverty, malnutrition, education and health are some major challenges for the Sustainability development of Sri Lanka [13]. Furthermore, it was noted that firms tend to disclose more on the performance indicators related to the social aspect as these are already regulated by the laws and regulations of the government [12].

Our findings on human and societal aspect reporting are further aligned with prior results [14, 15, 16]. Firms in developing countries persistently report on social problems rather than economic, environmental and corporate governance problems [14]. The social performance was more significant in Sri Lankan corporate sustainability practices. Social engagements in the forms of involvement in community projects, providing job training, contributing to charity, and improving employee education have been more substantial by the firms compared with environmental and economic sustainability practices. Further, social problems such as poverty and unemployment, which are considered typical issues in developing countries, are often predominated atop the environmental issues, explaining the dominance of human and societal aspect reporting in the corporate sustainability reporting of Sri Lanka [16].

Theme 2 – Economic aspect reporting

Economic aspect reporting is necessary for corporate sustainability reporting since shareholder wealth maximization is the ultimate objective of almost all business firms [17]. Reporting on the performance of the firms towards economic sustainability is essential in corporate reporting since the reports provide essential future directions of the firms for investor evaluations.

The second most frequently reported theme of the analysis was on economic aspect reporting with a reference percentage of 34.8%. Five nodes were identified under the economic aspect reporting, specifically; firms' commitment to (1) was improving the economic value generated, (2) improvement of infrastructure, innovations and digitalization, (3) improving the transparency of business processes, (4) gaining certifications and taking initiatives for best sustainability practices and (5) forming international and strategic partnership on sustainable development. Economic sustainability is supposed to be built on the effective management of three types of capital [11]. Financial capital includes equity and debt calculations. While; tangible capital consists of the physical machinery, land, stocks, intangible capital consists of reputation, technical knowledge, organizational procedures and innovations [11]. These concepts on economic sustainability are observed to be embedded in the five nodes related to the economic aspect reporting.

Nonetheless, in economic aspect reporting, a higher percentage of references is attributed to the firm's commitment to improving the economic value generated within the firm and community (15.40%), where the focused node was created referring to the eighth Goals of SDGs, which is "Decent work and economic growth". This goal

included the sub-nodes on the firm's commitment for enhancing the supply chain and employee productivity, community investment aimed for national economic growth, diversification of the product portfolios, effective management of the shareholder funds etc. It was observed that more emphasis is given to the reporting of improving the supply chain productivity and enhancing the employee and community based economic value generated. Our results in Table I are consistent with the most prioritized SDGs in corporate reporting processes as identified in KPMG 2020 and PwC 2018 reports [3, 4]. The reports stated that more than 50% of the global companies highly prioritize (72%) the "Decent work and economic growth" goal in their sustainability reports.

Moreover, considering the economic aspect reporting theme, our results are parallel to the prior study results. It was found that the second most common reporting content was on economic sustainability performance in the case of Chinese firms [14]. However, our results contrast to the findings of Bhatia & Tuli (2018), considering the sustainability reporting practises of emerging and developed countries. Economic aspect reporting was dominant in the study considering sustainability reporting of emerging economies in BRIC countries [7].

Theme 3- Environmental aspect reporting

Firm environmental performance is grounded on the commitment of the firms to conserve natural resources and ecosystem services. The industry is considered a living organism is referring to the term "industrial metabolism", where the industrial processes consume energy and resources, creating the anticipated output [11]. In the analysis, 6 nodes were identified on the content reported in corporate sustainability reports, namely; (1) Initiative for sustainable water management, (2) conservation and improving the efficiency of energy sources, (3) initiatives for resource consumption efficiency and efficient waste management practices, (4) initiatives for reduction of GHG emissions and carbon footprint, (5) sustainable practices in managing the water resources and beaches and (6) conservation and management of ecosystems and biodiversity. These nodes are significantly linked to the aforementioned natural resources and ecosystem services [11].

Sri Lanka is identified as a country with a rich sense of biodiversity and processing very delicate ecosystems in the world. However, the environmental reporting of Sri Lanka was found to be at a lower level in corporate reporting practices of Sri Lanka, parallel to the findings of the prior study [15]. It was stated that the lack of responsiveness to the severe environmental issues and the poor enforcement of the

environmental rules and regulations imposed by the government has attributed to the lesser reported content in corporate sustainability reporting in the Sri Lankan context [15]. The analysis revealed that most disclosures report on the efficient use of resources, waste management practice, and conservation of biodiversity in their annual reports related to the environmental aspect reporting in corporate sustainability reports considering the nodes. Most of the ecological aspect reporting content has included the firm's commitment to reducing the carbon footprint and GHG emissions, initiatives to enhance and maintain the forest and ecosystems, and reducing resource consumption. The main objective of the research was to identify the reported content in the corporate sustainability reports of Sri Lanka by conducting a thematic analysis.

Table 1. The major themes

Focused Nodes	Themes	Reference %
Improving the economic generated value within company and community	Economic Aspect Reporting	34.8
Improvements in infrastructure, innovation and digitalization		
Improving the transparency of business processes while reducing inequalities		

Certifications and initiatives on the best economic, social, environmental sustainability practices	Environmental Aspect Reporting	25.0
International or strategic partnerships on Sustainable development		
Initiatives for sustainable water management		
Conservation and improving the efficiency of energy sources		
Resource consumption efficiency & Waste Management practices		
Reduction of GHG emissions and carbon footprint		
Sustainable practices in managing the water resources and beaches		
Conservation & management of ecosystems and biodiversity	Human and Societal Aspect Reporting	40.2
Improving the primary living conditions of people		
Sustainable agricultural production & distribution processes		
Initiatives on Improving the health of people		
Satisfaction of the timely educational		

needs for improving productivity		
Minimize the gender inequality within the company and community		
Ensure fair and ethical work practices within the company		
		100

IV. CONCLUSIONS AND RECOMMENDATIONS

Along with most of the nation, Sri Lanka intensively practices GRI guidelines for corporate sustainability reporting [12]. Nevertheless, the diffusion of numerous guidelines, including the GRI, may differ from region to region, country to country due to the difference in understanding the established guideline concepts and choice of the firms on disclosing the performance in their sustainability disclosures. Thus, allegedly, it is expected that the reporting content on the sustainability reports may vary in different country settings even if the firms may state they use the same established sustainability reporting framework. The objective of our research was to explore the major reported themes in corporate sustainability reporting practices of firms in Sri Lanka. A qualitative thematic analysis approach was employed in this paper to identify the major reporting categories in corporate sustainability disclosures.

Sustainable Development Goals (SDGs) were used to identify the main categories and nodes in the reporting content in Sri Lankan Sustainability disclosures. In the analysis, 48 reporting categories were identified, with 17 focused nodes on the 17 SDGs. The node “improving the economic value within company and community” related to the SDG 8 was revealed to be more frequently reported in the corporate sustainability disclosures, parallel to the global findings of widespread reports [4, 3]. Consequently, the nodes “satisfying the timely educational needs” and “improving the health of employees and community” were shown to have a high reporting frequency. In contrast, the node “sustainable practices in managing the water resources or beaches” were observed to be given less priority.

Further, 17 focused nodes were classified into themes referring to the prior literature on the pillars of sustainability as “economic aspect reporting”, “human and societal aspect reporting,” and “environmental aspect reporting”. Human and societal aspect reporting consisted of 6 focused nodes, followed by 5 and 6 focused nodes for Economic aspect reporting and environmental aspect reporting, respectively. The highest references were quoted in the human and societal aspect reporting category (40.22%), followed by economic aspect (34.78%) reporting and environmental aspect reporting

(25%) categories. The results of the common themes were in line with the findings of prior literature in a developing country context [14, 15]. However, our primary focus was on determining the commonly reported categories in corporate sustainability disclosures. Thus, a significant emphasis is drawn on the nodes and sub-nodes created through the qualitative analysis.

Corporate sustainability reporting remains a voluntary practice among corporates in Sri Lanka. However, the adaption of reporting guidelines is more prevalent among the more prominent and multinational corporates. As SDGs open up new business opportunities globally, the firms should focus more on achieving the SDGs and communicating their sustainable performance through corporate sustainability reporting. As a universal reporting guideline is not yet formulated, it is desirable to identify a subtler guideline to report on firm sustainability performance. Presently, the study has identified the major themes reported through thematic analysis technique. Future research could be suggested to use more qualitative analysis techniques to enhance the quality of the study. The research findings would aid future researchers and industrial policymakers prepare new guidelines, particularly the Sri Lankan firms so that the investors could evaluate and compare how the firms have achieved sustainability and the firms' future

directions when forwarding their investments.

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FOCUS AREA
Food, Nutrition and Agriculture

A PRELIMINARY ESTIMATION OF DROUGHT TOLERANCE POTENTIAL OF SRI LANKAN COCONUT CULTIVARS THROUGH EVALUATION OF THE SEEDLING STAGE UNDER CONTROL ENVIRONMENT

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Abstract

Coconut is one of the major plantation crops in Sri Lanka with versatile uses. Climate change driven drought stress significantly threaten the coconut production in Sri Lanka. Therefore, breeding for drought tolerance has become a high priority research theme at present. In this study, a pot experiment was conducted with the objective of evaluating the Sri Lankan coconut cultivars for drought tolerance. Results revealed that, relative electrolyte leakage (REL) and chlorophyll parameters significantly varied with soil water content however, not among cultivars. There was significant ($P \leq 0.05$) negative correlation ($R^2 = -0.47$) between REL and soil water content (SWC). The Chl_a content and total chlorophyll content in leaf of coconut seedlings was significantly reduced with reducing soil water content. The visual drought score also varied significantly ($P \leq 0.05$) among coconut cultivars. Cultivar SLBD, SLGD and AS showed lower leaf drying under moisture stress condition compared to other cultivar tested. A comprehensive study needed to be carried out in future

to evaluate the coconut genotypes for drought tolerance.

Keywords: Coconut, Sri Lankan coconut cultivars, drought tolerance

I. INTRODUCTION

Coconut is one of the major versatile plantation crops that plays a significant role in food security and economy of people in many developing countries such as Sri Lanka (1). In Sri Lanka coconut is widely grown as plantations or home gardens mainly under rain-fed conditions.

There are three main coconut varieties found in Sri Lanka; Tall (Typica), Dwarf (Nana), and King Coconut (Aurantiaca) within each several forms are reported (2). In Sri Lanka, majority of the coconut lands are cultivated with Sri Lanka ordinary tall and about 30% of the coconut lands cultivated with improved Sri Lanka tall cultivar; the CRIC60. Dwarfs are not grown at commercial scale cultivation, except for beverage purpose (3). However, farmers' demand for hybrid coconut cultivars, developed by crossing dwarf with tall forms, are higher due to possession of desirable traits such

as early flowering and higher nut yields (4, 5).

Mainly three forms of dwarfs and two tall forms have been utilized by the Coconut Research Institute, Sri Lanka (CRISL) in coconut hybrid production. Sri Lanka Green Dwarf (SLGD), Sri Lanka Yellow Dwarf (SLYD) and Sri Lanka Brown Dwarf (SLBD) were used as dwarf forms while Sri Lankan Tall (TT) and one Philippine tall cultivar name San Raman were used as the tall forms.

Climate change is one of the most predominant challenges encountered in the 21st century which directly effects on the agricultural crop production (6). Among different abiotic stresses affecting on coconut, drought and high temperature are considered as major stress factors that cause negative impacts on nut yield (7, 8). As a result of the said challenges, breeding for drought tolerance, has become a high priority research area at present.

Evaluation of the existing varieties for drought tolerance is the first step of a long journey to produce a drought tolerant cultivars. However, evaluation of coconut cultivars for drought tolerance under field condition is challenging due to the practical difficulty in controlling the environmental factors and maintaining the soil moisture at conducive levels. Thus, pot experiments where seedlings are grown under greenhouse conditions are predominantly used as an alternative to screen perennial species for soil moisture stress (9).

In the current study, seedlings of the 12 Sri Lankan coconut hybrids and their respective parents were evaluated under simulated moisture stress conditions to identify their putative tolerance to drought.

II. MATERIALS AND METHODS

Planting material

Six hybrid cultivars, their parents and one of promising cultivar with high degree of drought tolerance (Table 1) were evaluated under simulated moisture stress conditions in the glass house of Plant Physiology Division of the Coconut Research Institute of Sri Lanka during the period of 2016-2017. Seven months old coconut seedlings were planted in 100 L plastic containers filled with a potting medium at a ratio of topsoil 2: coir dust 1. Simulated moisture stress was introduced by discontinuing the irrigation, two months after establishment under well-watered condition. Leaf samples from the youngest fully expanded leaf of each seedling were collected at every other week during the drought stressed period for biochemical analyses

Soil water content (SWC)

Soil samples from each pot (20 cm depth from the surface of potting medium) were collected. The weight of the fresh soil sample (FW) was measured, then the sample was dried at 80 °C for 48 h to obtain and dry weight (DW). The soil water content (%) was calculated using the following formula: $[(FW - DW)/DW] * 100$.

Table 1. Sri Lankan coconut cultivars used for the study

Coconut Varieties	Classification
Sri Lankan Green Dwarf (SLGD)	Dwarf form
Sri Lankan Yellow Dwarf (SLYD)	Dwarf form
Sri Lankan Brown Dwarf (SLBD)	Dwarf form
Sri Lankan Tall (SLT)	Sri Lankan Tall form
San Raman	Philippine Tall cultivar
CRIC65G (GDT)	Hybrid variety (Sri Lankan Green Dwarf x Sri Lankan Tall)
CRIC65Y (YDT)	Hybrid variety (Sri Lankan Yellow Dwarf x Sri Lankan Tall)
CRISL98 (TSR)	Tall x Tall Cross (Sri Lankan Tall x San Raman Tall)
CRISL2004 (GDSR)	Hybrid variety (Sri Lankan Green Dwarf x San Raman Tall)
TBD	Hybrid variety (Sri Lankan Tall x Sri Lankan Brown Dwarf)
SRBD	Hybrid Variety (San Raman Tall x Sri Lankan Brown Dwarf)
Ambakelle Special	Improved Tall variety by selection for yield stability over 20 years. (Samarasinghe et al., 2021)

Relative electrolyte leakage (REL)

Electrolyte leakage was measured according to Assaha et al (10). The fresh leaf samples were placed in vials with 100 ml of deionized water and shaken at 100 rpm for 24 hours at room temperature. The first electrolyte = $(EC1/EC2) \times 100$. The values were expressed as a percentage of maximum conductivity.

Chlorophyll content

Chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total chlorophyll (TC) contents in leaf tissues were analyzed using the method of Ashraf et al (11). Fifty milligrams of fresh leaf sample were blended with 5.0 mL 80.0% Acetone for 1 minute using a homogenizer and centrifuged for 10 minutes at 350 rpm.

leakage (EC1) was then recorded. Next, maximum conductivity (EC2) was obtained by placing the vials in the autoclave (Aster, Inc., Placentia, CA, USA) at 120 °C for 20 minutes. Electrolyte leakage was calculated as follows: electrolyte leakage

Thereafter, the absorbance of supernatant containing Chl_a, Chl_b were measured using a UV-visible spectrophotometer (Shimadzu UV-160, UV Visible Recording Spectrophotometer) at 662 nm, and 644 nm wavelengths and TC content was calculated as the sum of both Chl_a and Chl_b. A solution of 80% acetone was used as a blank. The chlorophyll concentration in (mg/g fresh weight) was then calculated using the method of Lichtenthaler and Wellburn (12).

Visual Scoring

Leaf drying of each seedling was visually scored at 14, 30, 37, 44, 51 and 55 days

after stress induction using a Visual Scoring System for coconut seedlings (Table 2) on a scale from 0 to 5 (13).

Table 2. Visual scoring system for coconut seedlings (12)

Leaf no	Percentage area of total leaf blade showed symptoms of drying					
2	<0.25	0.25	0.5	0.75	Totally dry	Totally dry
3	0	0.1	0.4	0.6	0.9	Totally dry
4	0	0.1	0.3	0.5	0.7	0.8
5	0	0.0	0.1	0.2	0.3	0.45
6	0	0.0	0.0	0.1	0.1	0.2
7	0	0.0	0.0	0.0	0.05	0.1
Score	0	1	2	3	4	5

Experiment design and statistical analysis

The experiment was conducted as a Completely Randomized Design (CRD) with six replicates (n=6). Parametric data (relative electrolyte leakage and chlorophyll content) were analyzed using Analysis of Variance (ANOVA) procedure and mean separation was carried out using Tukey's mean separation method. Pearson correlation analysis was performed for finding correlation between REL and SWC. Non parametric data (visual scoring) was analyzed through Kruskal-Wallis rank sum test. Statistical Tool for Agricultural Research (STAR) 2.0.1 software (IRRI) (14) was used for all data analysis.

III. RESULTS AND DISCUSSION

According to the results, drought induction leads to gradual depletion of soil water content reaching to 3.4% level after 55 days (Figure 1).

Figure 1 also shows the variation of Relative electrolyte leakage (REL) with time (days) after drought induction. Although the REL was not significantly increased over time at the beginning, it showed a rapid increase with reducing soil moisture content especially during the period from 45-47 days after the induction of drought.

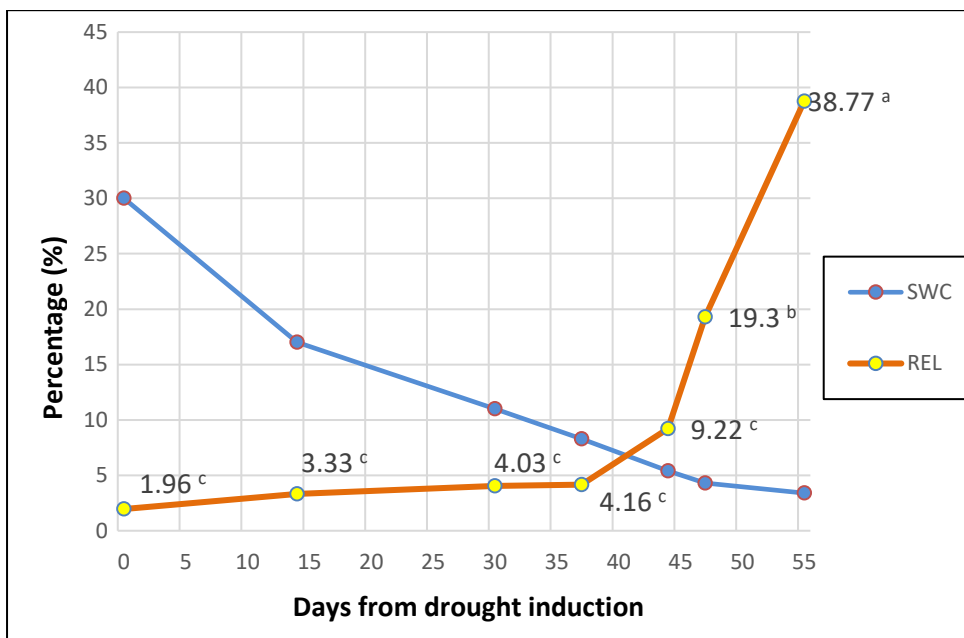


Figure 1. Variation of soil water content (SWC) and relative electrolyte leakage (REL) with time (days), after drought induction

As a result, there was a significant ($P \leq 0.05$) negative correlation ($R^2 = -0.47$) between REL and SWC.

REL is one of the most frequently used indicator for evaluating plant tolerance to drought and other abiotic stresses (15). Drought stress induced the cell membrane injury, leading to

intercellular ion efflux. REL value reflects the change of ion exosmosis, and thus the level of damage to cell membranes can be determined. The ANOVA showed that REL varied significantly only with soil water content but not with variety or variety x soil water content interaction (Table 3).

Table 3. ANOVA table for relative electrolyte leakage

Source	DF	Sum of Square	Mean Square	F value	Pr(> F)
Variety	11	2570.63	233.69	1.19	0.2986
Soil water content	5	34919.48	6983.90	35.58	0.0000
Variety x Soil water content	55	7670.64	139.47	0.71	0.9260
Error	144	28265.74	196.29		
Total	215	73426.49			

Chlorophyll is an essential pigment molecule for the capture and transfer of energy in the photosynthetic machinery (16). Degradation of Chlorophyll in moisture stressed palms is a general observation in plants subjected to water stress or reduced SMC. A significant ($P \leq 0.05$) variation of all chlorophyll related parameters was observed only with soil moisture level but not with other major factor (variety) or interaction between variety x soil water content. Chl_a content in leaf of coconut seedlings was significantly reduced with reducing soil moisture content (Table 4) which reveals that Chl_a content in leaf of coconut seedling was highly sensitive to moisture stress. Similar observations have been reported in other crops too (Olive; (17), Oil palm; (9)). In contrast to the observation on Chl_a, the degradation of Chl_b was not observed immediately with the reduction of SMC. However, a significant reduction was observed when

SMC was reduced below 8.3%. The change in TC was more or less similarly to that of Chl_a. However, Chl_a:Chl_b ratio in coconut leaf was reduced significantly ($P \leq 0.05$) only under extreme moisture stress (4.3% SMC) condition (Table 4).

Visual Scoring

The Kruskal-Wallis rank sum test showed the visual drought score varied significantly ($P > \text{Chi-Square} = 0.0121$) among coconut varieties. According to the mean of rank (Table 5), cultivar SLBD identified as most tolerant cultivar with lower leaf drying under moisture stress condition. Within dwarf group, SLYD showed higher leaf drying compared to other two dwarf cultivars. AS showed it's promising drought tolerant character with low mean rank compared to other two tall varieties.

Visual drought score is used as an alternative approach to determine plant drought tolerance (18).

Table 4. Chlorophyll a (Chl_a), chlorophyll b (Chl_b) total chlorophyll (TC) contents and Chl_a:Chl_b ratio in the leaf tissues of coconut seedling when subjected to moisture stress

No of days from drought induction	Soil water content (%)	Chl _a (mg g ⁻¹ FW)	Chl _b (mg g ⁻¹ FW)	TC (mg g ⁻¹ FW)	Chl _a :Chl _b
14	17	1.31 ^a	0.57 ^a	1.87 ^a	2.13 ^a
30	11	1.17 ^b	0.52 ^a	1.69 ^b	2.28 ^a
37	8.3	0.89 ^c	0.44 ^b	1.32 ^c	2.08 ^a
44	5.4	0.71 ^d	0.36 ^c	1.07 ^d	2.06 ^a
51	4.3	0.54 ^e	0.31 ^c	0.85 ^e	1.42 ^b

(Means with the same letter within a column are not significantly different at $P \leq 0.05$)

Table 5. Mean rank resulted by Kruskal-Wallis rank sum test for visual drought scoring of seedlings of different coconut cultivars under moisture stress conditions

Cultivar	Mean of Ranks
SLT	44.25
SLGD	20.28
SLYD	37.00
SLBD	11.67
SR	39.25
AS	28.90
YDT	54.67
GDT	26.92
TSR	37.08
GDSR	32.67
BDT	50.90
BDSR	44.08

Research findings with rice germplasm (19, 20) revealed that it is a reliable measure of tolerance for the estimation of oxidative damage in plants, and reflects dehydration of the plant tissue correlated with its relative water content. In rice, Standard Evaluation System (IRRI, 1996) was most frequently used for screening varieties for drought tolerance (20, 21). However, visual drought scoring in coconut is only applicable for seedling stages.

In the current study, only the leaf dryness (measured in terms of visual scoring) showed cultivar variation for drought stress. However, other two

parameters did not exhibit significant ($P \leq 0.05$) variation among cultivars. We infer that, evaluation of coconut cultivar for drought tolerance by only considering those parameters are inadequate. Detailed measurements of leaf gas exchange parameters; photosynthesis, respiration, stomatal conductance and transpiration rates are necessary for better understanding of drought tolerance capacity of different coconut cultivars.

IV. CONCLUSIONS AND RECOMMENDATIONS

Electrolyte leakage, chlorophyll degradation and leaf drying in coconut seedling were significantly related to the soil moisture content at which they are grown. Based on leaf drying, SLBD, SLGD, AS and hybrid variety GDT shown some drought tolerant ability compared to other cultivar tested. However, other two parameters did not give such variety level variation. Therefore, comprehensive study including measurement of major physiological and biochemical traits and molecular based study should be required for screening coconut genotypes for drought tolerance.

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EVALUATION OF DIFFERENT RICE VARIETIES FOR FOOD PREFERENCE HABIT OF LESSER BANDICOOT, *Bandicotabengalensis* UNDER CONTROLLED CONDITIONS

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Abstract

Rice is widely cultivated all over the world including Sri Lanka. Though maximum yield potential is expected from rice cultivation, it is limited by biotic and abiotic stresses. Apart from insect pests, rice cultivations are damaged by rats. In field conditions, *Bandicota bengalensis* is the most abundant species in Sri Lanka. Although evidence revealed that *B. bengalensis* has selective nature for their food preferences, it is not clear with different rice varieties. Therefore, this study was aimed to find the relationship between the food preference of rats and different rice varieties. The experiment was conducted in Rice Research and Development Institute, Bathalagoda under controlled conditions during the 2020 *Yala* and 2020/21 *Maha* seasons. Sixteen rice varieties (as treatments) were used with three replicates for each variety according to a CRD design and *B. bengalensis* was used as test rat species. Seven days after panicle initiation, 50 rats were introduced. The number of damaged tillers by rats were counted at 3, 5 and 7 days after rat

introduction. Damaged percentages were transformed into arcsine values and analyzed following ANOVA using SAS. The highest preference was shown for Bg403 followed by Bw367 and Bg379-2 which had no significant difference among them. The variety Bg360 was showed lower preference by rats followed by Bg251 and Bg310. The highest preference and lowest preference for rice varieties in *Maha* season were the same with data of *Yala* season. Higher preferable rice varieties by field rats have higher crop loss during both seasons. Therefore, preventive measures should be implemented during both seasons, especially for higher damaged varieties.

Keywords: Rice varieties, *Bandicota bengalensis*, Controlled conditions, Varietal preference

I. INTRODUCTION

Rice is the staple food of one-half of the world population [1] and 90% of the world rice production and consumption is done by the Asia region [2]. There are some constraints associated with rice cultivation that create some difficulties in reaching the full yield

potential. Reducing pest losses is a key strategy for increasing rice productivity [3]. Rodents also serve as essential vectors or reservoirs for several zoonotic diseases that affect humans, domestic animals, and other wildlife. The most abundant rat species in rice fields of Sri Lanka is *Bandicota bengalensis* [4] which feed predominantly on rice stems in tillering and booting stages. The damage percentage caused by *B. bengalensis* is associated with biotic and abiotic factors. However, these associations have not been studied much and published in Sri Lanka. Identification of such properties will help to implement more effective rodent management strategies in rice fields. Therefore, this study was carried out to find the preference of *B. bengalensis* for popular rice varieties, which are widely grown in Sri Lanka. Moreover, this study provides better insights into the management of *B. bengalensis*.

II. MATERIALS AND METHODS

This experiment was conducted at a plant cage in the Rice Research and Development Institute of Battalagoda during the 2020 Yala and 2020/21 Maha seasons. The rat species, *Bandicota bengalensis* was used which is the most common rat species found in the rice field in Sri Lanka. Sixteen rice varieties (as treatments) were used with three replicates for each variety according to a CRD design. Sixteen rice varieties were: Bg403, Bg366, Bw367, Bg360, Bg358, At362, Bg374, Bg379-2, Bg357, Bg352, MA 2, Bg251, At311,

At303, Bg306 and Bg310. The planting dates were adjusted to synchronize the panicle initiation stage of all the varieties. *B. bengalensis* collected in fields using Single-capture live traps (Cage traps) which are made from open material/wiremesh. Roasted coconut and unhulled paddy were used as baits which were placed inside the cage trap to attract the rats. After collecting them, species were identified and *B. bengalensis* was separated according to the Keys developed by [5]. The selected healthy male and female adults were kept inside a resting place prepared in the side of the experimental house maintained until use for experiment while providing the common food. The rats were starved twenty-four hours before rat introduction with supplying the water. Seven days after the panicle initiation, 50 starved rats were introduced to the cage. Daily observations were done to record the number of total tillers and the number of damaged tillers were obtained at 3, 5 and 7 days post rat introduction. The level of damage caused by rats was determined using the following formula.

$$\text{Damage \%} = \frac{\text{Number of damaged tillers}}{\text{Total number of tillers}}$$

Rat damage was distinguished by observing the 45-degree angle cut surface at the base of the tillers and panicles. In addition, both damaged and regenerated tillers were

considered as damaged tillers. Before analysis of the data, recorded percentage data were transformed into arcsine values and ANOVA was conducted using SAS statistical software [6]. Means were separated using Tukey's test where $P < 0.05$ to check for significant differences between treatments.

The ethical clearance was obtained from the Institute of Biology Sri Lanka (IOBSL) according to the Act of Parliament No. 22 of 1984. The registered registration number was ERC IOBSL 201 09 2019.

III. RESULTS AND DISCUSSION

The results indicated that the preference of *B. bengalensis* for selected rice varieties was significantly different under the controlled conditions in the 2020 Yala season. The first count ($F=410.23$, $p<0.0001$), second count ($F=425.68$, $p<0.0001$) and third count ($F=345.18$, $p<0.0001$) were significantly different in 2020 Yala season. The highest preference was shown for Bg403 in the first count. The next higher preference was shown for Bw367 and Bg379-2 which had no significant difference among them. The variety Bg352 had the next higher preference. There was no significant difference between Bg374 and At362, which served as fourth preferable varieties by the rats. The rice varieties At311, Bg357 and MA2 had no significant difference among them which appeared as the fifth preferable varieties by rats. There was no significant difference between Bg358,

At303, Bg300 and Bg366 which were less preferred by rats as next varieties. The variety Bg360 had low preference by rats but it was significantly higher than Bg310 and Bg251. There was no significant difference between Bg251 and Bg310 which showed the least preference by *B. bengalensis*. The damage percentage caused by field rodents during Yala season under controlled conditions is shown in Figure 1.1.

During Maha season, the varietal preference for selected rice varieties by *B. bengalensis* was significantly different in all three counts; first count ($F=316.83$, $p<0.001$), second count ($F=272.64$, $p<0.0001$) and third count ($F=402.52$, $p<0.0001$). The damage percentage caused by field rodents during Mahaseason under controlled conditions is indicated in figure 2.1. The highest preference was shown for Bg403 followed by Bw367 and Bg379-2 which had no significant difference among them. The variety Bg374 was the next preferred rice variety by rats followed by At311, Bg352 and Bg357 which showed no significant differences among them. Bg352, Bg357 and At362, did not show any significant difference. Apart from that, Bg357, At362 and At303 which did not show any significant difference had medium preference by rats. The next preferred varieties by rats were At362, At303 and Bg358 which had no significant differences. The next preferable varieties by rats were At303, Bg358 and MA2 which had no significant differences among them. The varieties

Bg366, Bg251 and Bg310 showed the least preference by rats, which had no significant differences among them.

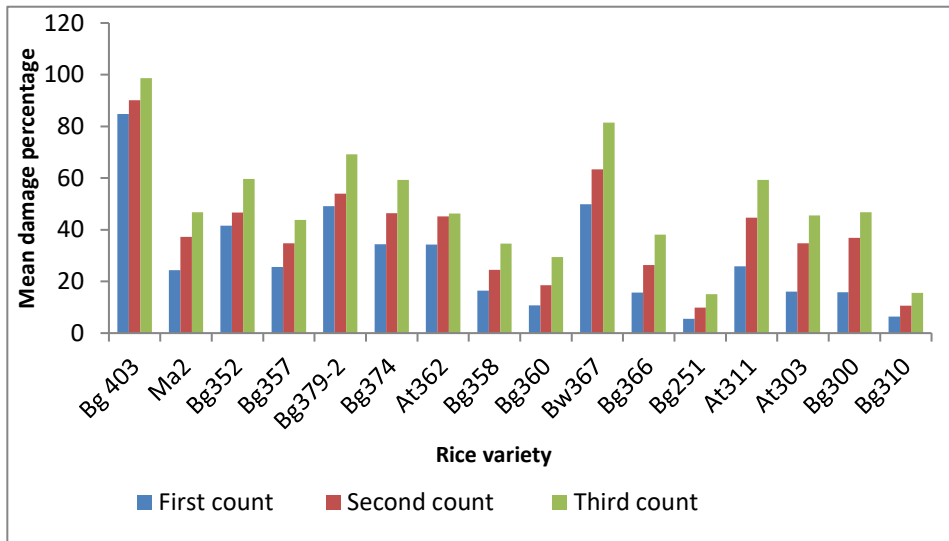


Figure1. Damage percentage caused by field rodents during *Yalaseason* under controlled conditions

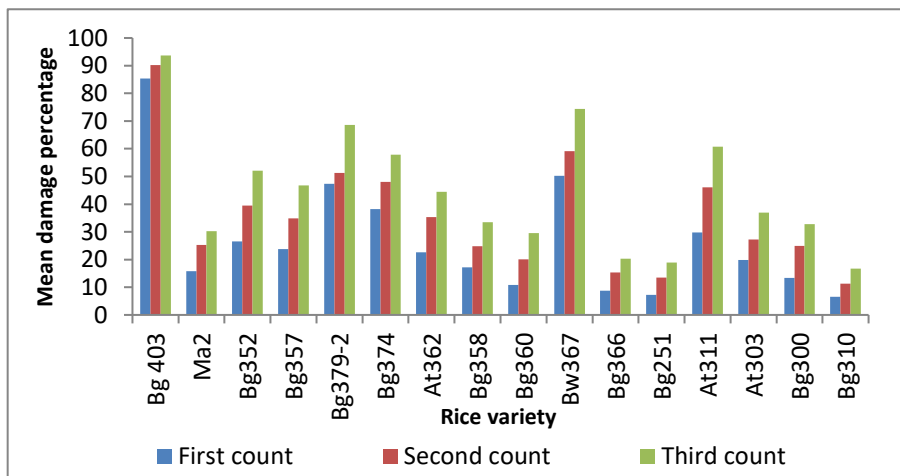


Figure 2. Damage percentage caused by field rodents during *Mahaseason* under controlled conditions

The rat species *B. bengalensis* had the highest preference on Bg403 rice varieties in comparison to the other varieties in both seasons under controlled conditions. The least preferable varieties were Bg366, Bg251 and Bg310, which were almost the same for both seasons as well.

According to [7], the positive correlation between tiller density and damage is important because this relationship tends to reveal the effect of rat damage on yield. The rat damage of the fields with a high density of tillers is severe in comparison to the fields having low-density tillers. This is also consistent with the well-known tendency of the feeding habits of the rats. In this context, they prefer to feed in good cover places and to avoid places where open places. Since different varieties may differ in grain size and the number of grains per tiller, factors would be expected to affect yield independently of tiller density.

In the tillering stage, *B. bengalensis* fed predominately on rice stems. Several studies revealed that the stomach compositions of rats towards the end of the tillering stage consist of reproductive parts of rice plants, especially immature rice flowers, not grains. During and end of booting, their stomach consisted of rice grains and flowers but little or no rice stems.

Changes in their preference for the number of different rice varieties may be due to the physical and chemical properties of the rice plants. Succulent levels of rice plants may have a direct impact on the rat damage because it is easy to incise by rats to take as food.

Moreover, this succulent level of rice plants may be due to the over-fertilization of nitrogen fertilizer and varietal characters. Apart from that, the composition of chemicals in rice culm may have an impact on rat attraction especially some volatile compounds, flavonoids and other chemical constituents. Some studies revealed that some of these quality parameters are intrinsically governed by physicochemical properties such as amylose and amylopectin [8], [9]. The impact of chemical substances and physical properties of different rice varieties should be evaluated for such preferences by rats.

IV. CONCLUSIONS AND RECOMMENDATIONS

The experiment revealed that Bg403 rice variety is the most preferred rice variety and Bw367 and Bg379-2 were the next preferred rice varieties for the *B. bengalensis* (Field rats) under controlled conditions. Higher preferred rice varieties by field rats have higher crop loss during both seasons. Therefore, preventive measures should be implemented during both seasons and especially for these varieties to protect them from rat damages. Though other varieties showed low damages by field rats, it may vary from time to time due to environmental conditions, varietal characters of rice plants, behavioural changes and genetic conditions of the rats. Additionally, the physical and chemical properties of rice plants may have an impact on rat damage. Therefore, further investigations are needed to identify the impact of the above factors of different rice varieties and rats on rat damages.

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MICROORGANISMS AS BIOFERTILIZERS - TRENDS IN SRI LANKAN CONTEXT

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Abstract

This article is a bibliometric analysis of Sri Lankan research on biofertilizers carried out from 2000 to 2021. The purpose of this article is to identify the trend of Sri Lanka researches within the domain of biofertilizers and make a foundation for future researches. The analysis is based on 55 research articles identified using the Google Scholar search engine. There is an increasing interest in biofertilizer researches over the study period. It was able to identify the trending crops used in biofertilizer researches and crops need to improve the yield by using efficient biofertilizer application. The minerals, which are mobilized using biofertilizers and minerals that need consideration for mineralization by biofertilizer, can be identified. Microbial species that have used in considered studies give an idea to elaborate future researches to develop efficient biofertilizers. The majority of considered studies have focused on major macronutrients of plants and leading crops in the Sri Lankan agricultural sector. The path is open for the investigation of efficient biofertilizers 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 biofertilizers.

Keywords: Bacteria, Biofertilizer, Fungi, Nutrients, Plant

I. INTRODUCTION

Microorganisms are highly diverse organisms in the environment that directly involve nutrient cycling in the biosphere [1]. Due to anthropogenic activities and some natural causes, microbial load and diversity in soil has decreased. Therefore, it is highly required to add microorganisms artificially to the soil as biofertilizers to increase crop production [2]. Biofertilizer is a product of viable microorganisms, which directly affect to plant growth and crop production through enhancing soil properties and nutrient availability [3].

Plant Growth Promoting Rhizobacteria (PGPR), Arbuscular Mycorrhizal Fungi (AMF) and Rhizobial Biofertilizers are the major three types of microbial biofertilizers. PGPR involve with N fixation, mineral solubilization including phosphates and inhibition of pathogen activities while AMF mainly involve with K and P solubilization. Rhizobial biofertilizers mainly apply for N fixation [4].

Biofertilizers improve soil health through mobilizing essential plant minerals such as N, P, K, Ca, Fe, Mg, Zn, Mn, forming soil aggregates to stabilize the soil structure, decomposing and transforming soil organic matter, Bioremediating to increase the usability of polluted soils and inhibiting the plant pathogenic action [4]. The application of biofertilizers enhances various growth parameters of plants at all stages of growth

compared to chemical fertilizer application alone [5]. Therefore biofertilizers are suitable as an alternative to chemical fertilizers [6].

In this paper, studies related to biofertilizers in Sri Lanka within the time period of 2000-2021 has analyzed and visualized the trends.

II. MATERIALS AND METHODS

A literature survey on biofertilizers in Sri Lanka for bibliometric analysis was done using Google Scholar in October 2021. The journal articles, conference proceedings and abstracts published in English between 2000 and 2021 were selected for the analysis. "Microbial biofertilizer in Sri Lanka" or "Microbial biofertiliser in Sri Lanka" was used as the title to search publications that resulted from 55 studies. The title of the study, name of the crops used for the study, nutrients; increased availability by biofertilizer, type of the microorganism, name of the microorganism, year and reference of all publications were recorded. Publication intensity and its trend regarding biofertilizer studies in the Sri Lankan context was analyzed. The trending plant varieties, basically focused nutrients and most possible types of microorganisms for biofertilizer studies in the Sri Lankan context were also analyzed.

III. RESULTS AND DISCUSSION

Summarization of identified 55 publications tabulated including title of the publication, name of the microorganism, year and reference (Table 01).

A. The volume of publications on biofertilizers in Sri Lankan context

Even the number of published articles shows fluctuations with time, it shows an

increasing trend and shows the increasing importance of biofertilizers over the years (Figure 1). Among all publications, ~96% have published in the latter half of the period considered for the analysis. The increasing number of biofertilizer research in Sri Lanka reflects the increasing concerns over environmental impacts of the usage of chemical fertilizers. The highest number of publications record in 2014 and World Bank records show the significant increment of fertilizer consumption (kilograms per hectare of arable land) in Sri Lanka between 2014 and 2015 [7].

B. Analysis of crop varieties used for biofertilizer studies

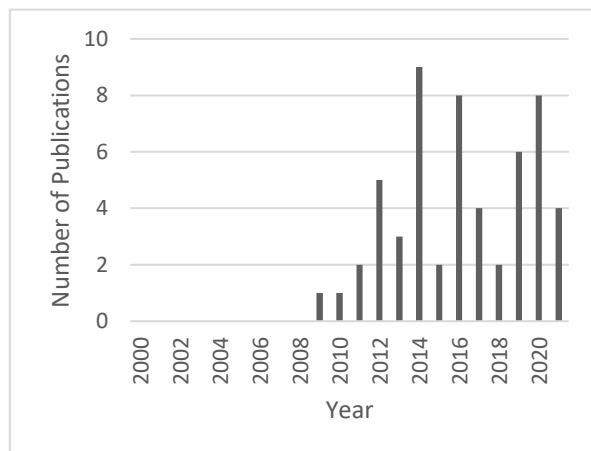
Among the analyzed 55 publications, the highest number of studies have used *Oryza sativa L.* as the crop model to investigate the impact of biofertilizers (Figure 2). That means increasing nutrient uptake of *Oryza sativa* for increasing yield is widely studied. *Oryza sativa L.* is the staple crop in Sri Lanka which responsible for food production. It is highly required to increase the food production parallel to the increasing population. Not only the food production, investigation of efficient biofertilizers for the staple crop of the country is directly affect for the environmental and public health of the country also.

C. Analysis of the nutrients; increased the availability by biofertilizer

A high number of identified publications have not mentioned about the nutrients mobilized by biofertilizers. Nevertheless, a high number of studies reveal that N,P,K; major macronutrients of plants have mobilized by biofertilizers while N is the frequent mineral among identified studies (Figure 3).

D. Analysis of the type of microorganisms used as biofertilizers

Bacteria, Fungi and Cyanobacteria are the frequently used biofertilizers in the Sri Lankan context, and combinations of microbial types are the best option other than the single organism because symbiosis relationships bring the best results. Biofilms of bacteria and fungi are the most common type used as biofertilizer in identified studies.



2021*- asterisk indicates data are till October 2021
Figure 1. The graph of Number of Publications Vs Year

Table 1. Summarization of identified publications

Title	Name of the Microorganism	Year	Reference
Propagation of <i>Bauhinia kockiana</i> Korth through stem cuttings as affected by maturity stage of cuttings and different biofertilizers	—	2020	[8]
Morphological and Molecular Identification of Cyanobacteria Isolated from Selected Paddy Fields at RRD1, Batalagoda	<i>Aphanothece</i> , <i>Chroococcus</i> , <i>Johannesbaptistia</i> , <i>Microcystis</i> , <i>Aphanocapsa</i> , <i>Synechococcus</i> , <i>Anabaena</i> , <i>Leptolyngbya</i> , <i>Pseudanabaena</i> , <i>Stigonema</i> , <i>Trichodesmium</i> , <i>Lyngbya</i> , <i>Oscillatoria</i> , <i>Nostoc</i>	2017	[9]
Emergence of Diverse Microbes on Application of Biofilmed Biofertilizers to a Maize Growing Soil	<i>Aspergillus</i> sp., <i>zorhizobium</i> sp., <i>Rhizobium</i> sp., <i>Acetobacter</i> sp., <i>Azotobacter</i> sp, <i>Azospirillum</i> sp.	2013	[10]
Co-Inoculation of <i>Pleurotus ostreatus</i> with Beneficial Bacteria Enhanced Substrate Utilization and Mushroom Production: An Implication for the Development of a Novel Bio-Fertilizer	—	2016	[11]

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Microbial aided leaching of Potassium from Sri Lankan Feldspar	<i>Bacillus mucilaginosus</i> , <i>Bacillus cereus</i> , <i>Aspergillus</i> sp.	2012	[12]
Potential of Biofilm Biofertilizer Application in Paddy Soil Carbon Sequestration in Sri Lanka: An Economic Feasibility Analysis	–	2020	[13]
Effect of Biofilm Biofertilizer on the Performances of Native Plant Species in degraded grasslands at Knuckles Forest Reserve, Sri Lanka	–	2020	[14]
Effect of fungal-bacterial biofilms on tuberization of potato (<i>Solanum tuberosum</i> L.)	–	2014	[15]
Biofilmed Biofertilizers for Rhizo-Remediation and Consumer Health-Friendly Potato Production	<i>Bacillus pumilus</i> , <i>Bradyrhizobium japonicum</i> , <i>Bacillus subtilis</i> and <i>Trichoderma harzianum</i>	2020	[16]
Variation of Biochemical Expressions of Developed Fungal-Bacterial Biofilms over their Monocultures and its Effect on Plant Growth	<i>Colletotrichum</i> sp., <i>Azotobacter</i> sp.	2013	[17]
Rice root interactions with a developed cyanobacterial biofilm and its monocultures	<i>Calothrix</i> , <i>Cylindrospermum</i> , <i>Nostoc</i>	2014	[18]
Developed Fungal-Bacterial Biofilms Having Nitrogen Fixers: Universal Biofertilizers for Legumes and Non-Legumes	<i>Acetobacter</i> , <i>Azotobacter</i> , <i>Bradyrhizobium</i> , <i>Rhizobium</i> , <i>Acremonium</i> sp.	2015	[19]
Effectiveness of Fungal Bacterial Interactions as Biofilmed Biofertilizers on Enhancement of Root Growth of <i>Hevea</i> Seedlings	<i>Bacillus</i> sp., <i>Penicillium</i> spp., <i>Aspergillus</i> spp., <i>Trichoderma</i> spp.	2014	[20]
Availability and leaching of nutrients after biofilm biofertilizer applications into a Red Yellow Podsolc soil		2014	[21]
Effect of biofilmed biofertilizer on plant growth and nutrient uptake of <i>Hevea brasiliensis</i> nursery plants at field condition	<i>Bacillus</i> spp., <i>Aspergillus</i> spp.	2018	[22]
Study on soybean (<i>Glycine max</i>) root growth performance under microbial inoculation, micorrhizal association and chemical fertilizer application	<i>Bradyrhizobium japonicum</i>	2011	[23]
Comparison of different organic amendments, biofertilizers and synthetic fertilizer on seedling growth of some dry zone forest species in Sri Lanka	<i>Arbuscular mycorrhizae</i>	2014	[24]
Influence of Biofertilizer Application on Growth, Yield and Quality Parameters of Jasmine (<i>Jasminum Auriculatum</i>)	<i>Azospirillum</i> , <i>Pseudomonas striata</i> , <i>Pseudomonas fluorescens</i> , <i>Trichoderma viridae</i> .	2014	[5]

Efficacy of single and combined application of <i>Trichoderma</i> spp. and <i>Pseudomonas fluorescens</i> along with bio-fertilizer (Arbuscular Mycorrhizae - AM) on growth of nursery plants of black pepper (<i>Piper nigrum</i> L.)	<i>Trichoderma</i> spp., <i>Pseudomonas fluorescens</i>	2016	[25]
Effect of Biofilm Biofertilizer and Chemical Fertilizer Application Practices on Growth and Endophytic Bacterial Count of Rice (<i>Oryza sativa</i> L.)	Endophytic bacteria	2019	[26]
Development of Microbial Biofertilizer for Tomato (<i>Solanum Lycopersicum</i>)	–	2019	[27]
Effect of soil applied organic amendments and microbial inoculant on the improvement of growth and yield of soybean (<i>Glycine max</i> L.)	<i>Bradyrhizobium japonicum</i>	2020	[28]
Efficiency of Biofilm Biofertilizer in Increasing Plant Growth Parameters of Rice Cultivation In Sri Lanka	–	2019	[29]
Influence of mycorrhizae and inorganic fertilizer on plant growth and yield components of two sri lankan traditional rice accessions (<i>Oryza sativa</i> L)	commercially available mycorrhizae	2016	[30]
Evaluation of Different Carrier Substances for the Development of an Effective Pelleted Biofertilizer for Rice (<i>Oryza sativa</i> L.) Using Co-inoculated Bacteria and Arbuscular Mycorrhizal Fungi	<i>Azospirillum</i> sp., <i>Pseudomonas fluorescens</i> , arbuscular mycorrhizal fungi	2020	[31]
<i>Azorhizobium caulinodans</i> ORS 571– <i>Aspergillus</i> spp. biofilm in the presence of flavonoid naringenin: An extremely effective association for rice root colonization with a definite future as a nitrogen bio-fertilizer	<i>Azorhizobium caulinodans</i> ORS 571, <i>Aspergillus</i> spp.	2017	[32]
Biofilm biofertilizer can reinstate network interactions for improved rice production	–	2021	[33]
Isolation of phosphate solubilizing bacteria for production of biofertilizer	–	2012	[34]
A biofilmed bio fertilizer for tomato	–	2013	[35]
Comparison of the effects of organic fertilizers with inorganic fertilizers on the growth of eight months old coconut seedlings and the nutrient availability and soil microbial activity of soils	<i>Azotobacter chroococcum</i> , <i>Pseudomonas fluorescens</i>	2010	[36]
Effect of Biofilm Biofertilizer on Availability of Soil Diazotrophs, Plant Endophytic Diazotrophs and Increasing of Grain Yield in Rice (<i>Oryza sativa</i>) Cultivation of Sri Lanka	Diazotroph bacteria	2019	[37]
Nutritional properties and hydrolyzing rates of rice grown with biofilm bio-fertilizer (bfbf)	–	2021	[38]

Effect of a Non-native Biofilmed Biofertilizer for Rice in the Eastern Province of Sri Lanka	–	2012	[39]
Effect of biofilmed biofertilizer on rice growth in the native soils of the component microbes	–	2015	[40]
Organic Fertilizers and Biofertilizers to Improve Growth, Yield and Nutrient Quality of Soybean (<i>Glycine max</i> L.)	–	2016	[41]
Biofilm biofertilizers for incorporating biodiversity benefits and reducing environmentally harmful subsidies in agriculture	–	2017	[42]
Developed microbial biofilms can restore deteriorated conventional agricultural soils	<i>Acetobacter</i> spp., <i>Azotobacter</i> spp., <i>Rhizobium</i> spp., <i>Bradyrhizobium</i> spp., <i>Colletotrichum</i> sp.	2011	[43]
Biochemical Expression of Exudates of a Fungal-Bacterial Biofilm During Growth and Maturation	<i>Azotobacter species</i> and <i>a Colletotrichum fungal</i>	2012	[44]
Fungal-bacterial biofilms improve early vegetative growth of strawberry (<i>Fragaria x ananassa</i>) over their monocultures	–	2014	[45]
Profitability of strawberry (<i>Fragaria ananassa</i>) production with biofilmed biofertilizer application	<i>Aspergillus</i> sp., <i>Enterobacter</i> sp.	2019	[46]
Functional heterogeneity of metabolites excreted by fungal and bacterial biofilms and their effects on seedling growth	<i>Aspergillus</i> sp., <i>Enterobacter</i> sp.	2020	[47]
Biofilmed biofertilizers for improved quality and quantity of strawberry (<i>Fragaria ananassa</i>) under field conditions	<i>Aspergillus</i> sp., <i>Enterobacter</i> sp.	2021	[48]
Isolation and Identification of Culturable Cyanobacteria from Paddy Soils of the Intermediate Zone of Sri Lanka	<i>Aphanothece</i> , <i>Chroococcus</i> , <i>Johannesbaptistia</i> , <i>Microcystis</i> , <i>Anabaena</i> , <i>Pseudanabaena</i> , <i>Nostoc</i> , <i>Stigonema</i> , <i>Trichodesmium</i>	2016	[49]
Assessing anaerobic bacteria survival in different microbial inoculant preparations to use in sustainable paddy cultivation in dry zone of Sri Lanka	–	2014	[50]
Microbial respiration and nitrogen mineralization in soil amended with different proportions of vermicompost and coir dust	–	2009	[51]
Bio fertilizer: microbial inoculant from agricultural waste	–	2016	[52]
Biofilmed biofertilizers can replace bulky organic fertilizer handling in organic rice cultivation of Sri Lanka	–	2012	[53]

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Development of Nitrogen Bio fertilizer and Its Effect on Growth of Capsicum (<i>Capsicum annum</i> L.)	–	2018	[54]
Reinstating Soil Biodiversity: The Key for Converting Degraded Lands to Sustainable Systems	–	2016	[55]
Effect of sugars, amino acids, hormones and microbial biofilm exudates on dormancy breaking of culturable soil microbial seed bank	–	2017	[56]
Isolation and Characterization of Rhizobia from Leguminous Plants and Determining their Plant Growth Promoting (PGPR) Traits	<i>Bradyrhizobium</i> spp.	2016	[57]
Effect of bacterial biofertilizers, native arbuscular mycorrhizal fungi and soil amendments on soil and grain phosphorus availability of flooded rice in dry zone, Sri Lanka	<i>Azospirillum</i> sp., <i>Pseudomonas</i> sp. <i>Bacillus</i> sp., Arbuscular mycorrhizal fungi	2020	[58]
Growth, Yield and Seed Nutrient Quality of Soybean (<i>Glycine max</i> L.) as Affected by Organic, Biofertilizer and Synthetic Fertilizer Application	<i>Bradyrhizobium</i> spp.	2019	[59]
Effect of Different Potting Mixtures on Growth and Yield of Soybean (<i>Glycine max</i> L.) and Soil Microbial Activity	–	2014	[60]
Multi-phasic Nitrogen Fixing Plant Growth Promoting Rhizobacteria as Biofertilizer for Rice Cultivation	<i>Bacillus</i> sp., <i>Pseudomonas</i> sp.	2021	[61]

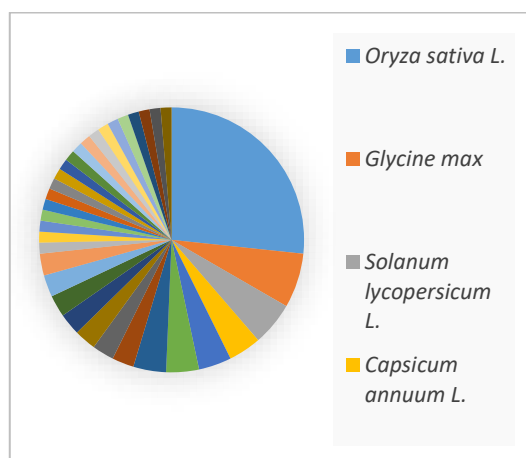


Figure 2. The graph of reported plant varieties used for biofertilizer studies in Sri Lanka, 2000-2021

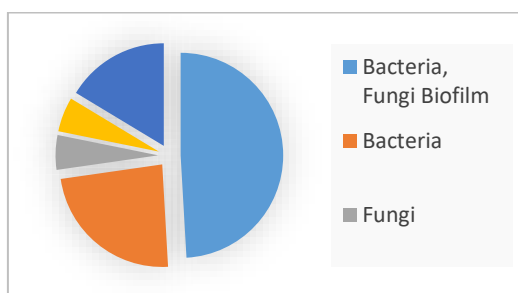


Figure 3. The graph of nutrients; increased the availability by biofertilizers according to the published data in Sri Lanka, 2000-2021

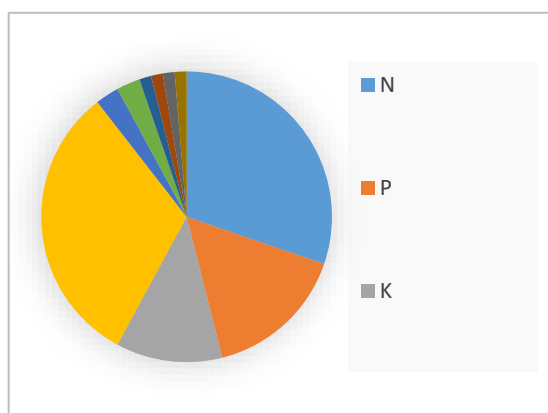


Figure 4. The graph of microbial types used in biofertilizers

IV. CONCLUSIONS AND RECOMMENDATIONS

The analysis of published 55 studies within the considered time (2000-2021) shows that increasing interest in the field of biofertilizers. *Oryza sativa* L.; the staple crop that fulfils the food requirement in Sri Lanka, is the frequent crop variety used in biofertilizer studies during the period. Furthermore, *Camellia sinensis* and *Hevea brasiliensis* are the major economic crops in Sri Lanka. This trend is important because leading crop varieties in Sri Lankan agricultural sector has been studied for increasing yield with biofertilizers. The majority of studies has focused on N, P, K; Major macronutrients of plants to mineralize using biofertilizers. As well as it is better to focus on essential micronutrients because those minerals are also necessary for better performance of plants even at low doses. In addition, results show that the

combination of bacteria and fungi gives better results than the single-use.

To conclude, biofertilizer is an emerging field of research globally, and it has been shown in the Sri Lankan context. Biofertilizer studies are not limited to agriculture and food production, it is expanded through the vast area including environmental science, microbiology, biotechnology, sustainability, mineralogy and chemistry. Therefore, conducting credible studies will affect the economic development of the country. The findings of this paper will make a foundation for future researches in biofertilizers.

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DRY SPELL CHARACTERISTICS IN SEVANAGALA, SRI LANKA AND ITS IMPACT ON SUGARCANE CULTIVATION

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Abstract

Daily precipitation data available in the agrometeorological station of the Lanka Sugar Company, Sevanagala Factory for 37 consecutive years has been used to estimate the frequencies of consecutive dry day events and to assess its effect on the sugarcane cultivation program. The results revealed that the events longer than 60 consecutive dry days can be expected in every 5.3 years interval. Frequency of occurrence of 10-19 and 30-39 consecutive dry day events have increased significantly during sugarcane harvesting seasons. Even though, the droughts occurrence during the harvesting season improves sugar accumulation in cane stalks, lengthy dry spells caused high negative impacts on germination, tillering and growth of young sugarcane plants which were planted during the immediately passed planting season. Therefore, the plantation management plan should be prepared to improve supplementary irrigation facility and re-adjusting the cropping calendar to skip immature crops from lengthy consecutive dry day events.

Keywords: Rainfall, Dry spells, Sugarcane, Sri Lanka

I. INTRODUCTION

United States Agency for International Development (USAID) reported that the length of the drought in Sri Lanka has increased significantly in recent years due to climate change [1], and change of the rainfall patterns adversely affect many crops grown in the dry zone of Sri Lanka [2,3,4,5]. The rain-fed sugarcane is one of such vulnerable crop in Sri Lanka [6,7,8]. According to the previous studies, rainfall is the most important climatic factor for rain-fed sugarcane production in Sri Lanka, due to lack of any other source of water for the rain-fed sugarcane plantations [9]. Capillary water from groundwater table or springs is restricted in most rain-fed sugarcane plantations in the Sevanagala area [10]. Therefore, the amount of sugarcane that can be harvested in rain-fed plantations is greatly related to the amount of rainfall received and its distribution pattern [7,12]. In addition to significant yield losses of sugarcane crop [13], cane quality also reduces due to effect of prolonged drought. Therefore, any change in the expected rainfall will affect the entire production line of the sugarcane industry.

Analysis of consecutive dry day events is a widely used analytical technique in characterizing short and long-term drought [14,15,16,17]. The length of consecutive dry day events is calculated by summing up of all consecutive dry days that are not interrupted by rainfall. Data on the frequency of occurrence of consecutive dry day events are useful in planning the sugarcane planting schedule to minimize the adverse effects of such dry spells. This paper describes the drought patterns, frequency and their effect on the current planting and harvesting schedule of the sugarcane plantation in Sevanagala, Sri Lanka.

II. MATERIALS AND METHODS

Study area

The study was conducted in sugarcane plantation in Sevanagala, Sri Lanka (latitude from 6° 26'36.53" N to 6° 20'4.35"N and longitude from 80° 50'31.94"E to 80° 58'10.23" E). The area is located on the western border of Monarajala district in DL1b agro-ecological zone [18]. The average annual temperature of the area is about 28.6°C and the average relative humidity is 72.6 % [6,12,19]. The class A pan evaporation is around 4.5 mm/day [12]. Rainfall has a bimodal distribution pattern with two peaks in April and November. Accordingly, minor rainfall season extends March to May (Yala season) and major rainfall season from October to January in Maha season [20,21]. Maha season is the main growing season of sugarcane in Sevanagala and planting is often done in between October to

November. In few locations, a minor planting season is also practiced during Yala season from March to April period. Main harvesting season of sugarcane in Sevanagala extends from May to October, while minor harvesting season experiences from January to March [25,19].

Data

Daily rainfall data for 37 consecutive years from 1984 to 2020 available in the agrometeorological station installed at the center location of the sugarcane plantation in Sevanagala (latitude: 6° 23'46.93"N; longitude: 80° 54'45.54" E) were taken for the analysis.

Dry day selecting criteria

Different authors have used various threshold levels to determine dry days. Most climatological studies considered a day with a daily rainfall of less than 0.1 mm as a dry day, and in agricultural studies, less than 2.5 mm [22,14]. Threshold level of 2.5 mm daily rainfall is often applicable in most agricultural crops as most of lite rainfalls are getting evaporated without contacting with the root zone soils due to adsorption and resistance of the crop canopy foliage [11,23]. Therefore, day that daily rainfall is less than 2.5 mm was considered as a dry day in present analysis.

Duration of consecutive dry day event

The period between two successive rainfall days which recorded daily rainfall is greater than 2.5 mm was taken as the duration (number of day) of the consecutive dry day events. If

the consecutive dry day event continues in between two adjacent months, it was treated as one successive dry day event, and the corresponding month of that event was selected as the greatest number of dry day experienced month. This was simply determined by selecting median date of the data series of the particular dry day event. If a consecutive dry day event has extended between two years i.e. month of December and January in between consecutive two years, the corresponding year was determined as similar logic described above.

Return period

Consecutive dry day events were grouped according to the length of the events as 10-19 day, 20-29 day, 30-39 day, 40-49 day, 50-59 day and longer than 60 days. The return period of consecutive dry day event was calculated as per the equation 1.

$$T = \frac{1}{p} \quad (01)$$

Where, T = Return period (years), p = Probability of occurrence.

the events of a consecutive 20-29 dry day event occur 1.7 times per year.

It is evident that (Table 1), the events with 30-39 consecutive dry days have a tendency to occurs in every 1.3 years. In addition, chance for occurrence of 40-49 and 50-59 consecutive dry day events in Sevanagala are once per every 4.1 years and once per every 12.3 years respectively. Furthermore, consecutive dry day events with 60 days or more than 60 day can be expected in every 5.3 years. This proves that the Sevanagala rain-fed sugarcane crop is exposed to a prolonged severe drought once in 5.3 years. Due to such a prolonged drought, crop failures are often expected and should be replanted at 5-year intervals. This finding is consistent with the average of number of ratoon cycles crop-up in most farmers filed in Sevanagala rain-fed sector (purs.com with Plantation Extension Manager, Lanka Sugar Company, Sevanagala Factory, 2019).

III. RESULTS AND DISCUSSION

Occurrence of consecutive dry day events

The average annual rainless day (<2.5 mm) in Sevanagala is approximately 286 days. Table 1 lists the details of consecutive dry day events occurred in Sevanagala during 1984 and 2020 period. The results indicate that the consecutive dry day events that has a length of 10-19 day is likely to occur at the rate of 6 times per year. Similarly,

Table 1. Number of dry day events experienced in each year in Sevanagala corresponding to 10-19, 20-29, 30-39, 40-49, 50-59 and > 60 consecutive dry days

Year	Number of dry days	Number of wet days	Events count (Event's length in days)						Event's totals
			(10-19)	(20-29)	(30-39)	(40-49)	(50-59)	(> 60)	
1984	279	87	6	0	0	0	0	1	7
1985	273	92	5	2	0	1	0	0	8
1986	293	72	4	1	5	0	0	0	10
1987	308	57	1	4	0	0	0	1	6
1988	285	75	7	2	0	0	0	0	9
1989	302	63	7	1	2	0	0	0	10
1990	288	77	6	2	1	0	0	0	9
1991	279	86	8	2	0	0	0	0	10
1992	295	71	6	2	0	0	0	1	9
1993	287	78	7	2	0	0	1	0	10
1994	280	85	8	1	1	0	0	0	10
1995	284	81	7	0	0	0	0	0	7
1996	291	75	10	2	0	0	0	0	12
1997	273	92	6	2	0	1	0	0	9
1998	291	74	7	4	0	0	0	0	11
1999	290	75	3	4	0	0	1	0	8
2000	289	77	8	1	1	0	0	0	10
2001	294	71	5	3	0	0	0	1	9
2002	299	66	3	1	1	1	1	0	7
2003	296	69	4	2	0	1	0	1	8
2004	287	79	7	1	1	0	0	0	9
2005	278	87	2	2	2	0	0	0	6
2006	267	98	7	2	1	0	0	0	10
2007	294	71	5	2	1	0	0	0	8
2008	279	87	8	1	0	1	0	0	10
2009	269	96	6	3	0	1	0	0	10
2010	277	88	9	0	2	0	0	0	11
2011	275	90	10	0	1	0	0	0	11
2012	277	89	6	2	1	0	0	0	9
2013	285	80	6	1	2	0	0	0	9
2014	290	75	4	0	1	1	0	1	7
2015	276	89	7	2	1	0	0	0	10
2016	311	55	4	0	1	1	0	1	7
2017	285	80	9	1	1	0	0	0	11
2018	290	75	5	1	1	1	0	0	8
2019	267	98	7	1	1	0	0	0	9
2020	300	66	3	5	1	0	0	0	9
Total	10,583	2,926	223	62	29	9	3	7	333
AVG	286±1.8	79.1±1.8	6.0±0.3	1.7±0.2	0.8±0.2	0.2±0.1	0.1±0	0.2±0.1	9±0.2
Return period			0.2	0.6	1.3	4.1	12.3	5.3	

Occurrence of consecutive dry day events during planting season

In general, 96 planting days per year are scheduled for planting operations while harvesting operations have about 247 days per year. Table 2 lists the details of the planting and harvesting season practiced in Sevanagala.

Table 2. The duration of the planting and harvesting season practicing in Sevanagala sugarcane plantation

Agronomic practice	Time period
Minor planting season	15 th march – 30 th April
Major planting season	1 st October – 15 th November
Minor harvesting season	15 th January – 31 st of March
Major harvesting season	15 th may – 31 st October

Source: pers. com, Lanka sugar, Sevanagala (2019).

The variation of number of occurrences of consecutive dry day events corresponding to 10-19, 20-29, 30-39, 40-49, 50-59 and > 60 day during sugarcane planting seasons and harvesting seasons are shown in the Table 3. The minor planting season has experienced 10-19 and 20-29 consecutive dry day events at a higher frequency than the major planting season reaching up to 100% and 75% respectively. Furthermore, dry day events of more than 30 consecutive days are experienced once in every 6.1 years during the minor planting season. Nevertheless, the analysis has

exhibited that the minor planting season will face a drought of more than 60 consecutive dry days once in 37 years. It is evident that the sugarcane planted in March-April has the risk of germination failures compared to October to November season. Therefore, it is necessary to introduce a supplementary irrigation program for the minor planting season.

Occurrence of consecutive dry day events during harve-sting season

Dry weather conditions are needed for maturing of sugarcane to accumulate more sucrose in the stalks [24,14]. Usually in the irrigated crop, the water supply should be cut down 30-60 days prior to harvesting.

The minor harvesting season experiences longer than 30 consecutive dry day events in every 2.1 years, while the major harvesting season being once in 0.6 years, which is 270% higher than that of the minor harvest season. Therefore, in order to obtain more sugar recovery at the factory, sugarcane harvesting during the January-March period should be avoided when and wherever possible [24,14]. However, an increase in the occurrence and frequency of prolonged drought during the harvesting season may adversely affect young sugarcane plants which were planted in immediate past planting season in terms of germination, tillering and growth.

Table 3. Occurrence of consecutive dry day events during the sugarcane planting and harvesting seasons

Length of the dry day event (days)	Planting season				Harvesting season			
	Minor		Major		Minor		Major	
	Event count	T	Event count	T	Event count	T	Event count	T
10-19	42	0.9	21	17.6	72	0.5	135	0.3
20-29	7	5.3	4	92.5	32	1.2	61	0.6
30-39	2	18.5	0	n.d	11	3.4	35	1.1
40-49	2	18.5	0	n.d	5	7.4	13	2.8
50-59	1	37.0	0	n.d	1	37.0	9	4.1
>60	1	37.0	0	n.d	0	n.d	6	6.2

n.d = not detected, T = Return period (Years)

IV. CONCLUSIONS AND RECOMMENDATIONS

In the sugarcane plantation site of Sevanagala Sri Lanka, the consecutive dry day events longer than 60 consecutive days can be expected in every 5.3 years. Nevertheless, probability of occurrence of 10-19 and 20-29 dry day events during minor planting season falling on March to April are higher than 100% and 75% respectively, compared to the major planting season (October to November). Therefore, it is recommended that to commence a supplementary irrigation program for the minor planting season. It is also recommended that the harvesting schedule to be planned in May to October to avoid enough continuous dry spells for proper maturation and high sucrose accumulation in the cane stalks.

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SHORT TERM EFFECTS OF STRAW MANAGEMENT PRACTICES ON THE PHYSICO-CHEMICAL SOIL PROPERTIES

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Abstract

Rice straw is considered as an abundant agricultural byproduct and developing viable straw management options in the dry zone farmlands may have profound effects on sustainable paddy production. The study was designed to assess the direct impacts on physio-chemical soil properties in sandy clay textured soil. Field experiment at Agrotech park Malwatta was carried out with three different straw management options viz; mulching (T1), burning (T2) and soil incorporation (T3) along with control (without straw application). Subsequently, fourteen days after straw imposition, soil samples collected from 10-15 cm deep and analyzed for soil properties and measured soil moisture, pH, electrical conductivity and for the carbon content of organic matter. The study's findings revealed that mulching with straw (T1) significantly increased the soil properties including moisture content (11.17%), pH (4.98), electrical conductivity (137 μ S/cm) and the organic carbon (2.08%) in short terms compare to control (7.55, 4.54, 0.94, and 1.05% respectively). Meanwhile burning of straw reduced soil moisture drastically (7.22%), electrical conductivity (0.12 μ S/cm) and organic carbon (1.66%). Furthermore, followed by mulching, straw incorporation (T3) into soil also exerts greater improvement in soil properties in short term than control treatment in the above sandy clay textured

soils. It was observed that mulching with straw improved soil qualities, including moisture content. Meanwhile, burning straw severely reduced soil moisture. The study recommends mulching as suitable management option in sandy clay textured soil and to avoid burning to maintain soil health.

Keywords: Burning, Mulching, Rice straw, Soil incorporation, Soil properties

I. INTRODUCTION

Solid waste generation and its environment friendly management are one of the environmental challenges in the present days. Crop residues are notable one and become menace unless managed properly. Straw is carbon-rich energy source with considerable levels of nitrogen, phosphate, potassium, and other nutrients essential for crop growth. The production of rice straw depends on rice varieties, soil fertility and climate. Rice straw management is critical, especially in Asia, which produces 90% of the world's rice. A variety of straw management options are available in terms of improvement soil and bioenergy production as well as materials production for industrial use. Each option has its merits and demerits.

Rice straw increases soil quality through increasing nutrient cycling and soil organic carbon retention, both of which promote

soil fertility. However, to keep up with their farms, farmers burn significant volumes of crop straw during production process, which results nutrient loss, soil organic C degradation, and decline in the presence of beneficial soil biota.

Ampara is one of the paddy growing districts in Sri Lanka. In 2019/2020 Maha and Yala respectively i contributes 11%, 15% of total production of the country [1]. Out of the total extents cultivated in 2019/2020 Maha and Yala respectively it contributes 6% and 13% of the fields were subjected to paddy burning in Sri Lanka [1].

Meanwhile, farmers are with lack of knowledge about negative effects of burning on environment as well as the short-term effects of other management options as they believe other management options' benefits could be attained in long terms and they have no such long time between the successive cropping cycles. Furthermore, literature available on direct effects of straw management options also is limited according to the best of authors' knowledge.

A study was conducted to

- determine the direct impact of straw management strategies on soil properties.
- understand the effects of burnt paddy straw as an amendment on the physicochemical properties of soil.
- determine the most effective straw management practices for improving soil properties.

II. MATERIALS AND METHODS

A. *Experimental site & design*

An open field experiment was conducted in Agrotech Park Malwatta in the Samanthurai Divisional Secretariat division of the Ampara district in Sri Lanka's Eastern Province (07° 19' 17.30" N, 81° 43' 56.19" E) at a height of 30 meters above sea level.

The field experiment used a randomly complete block design (RCBD) with four treatments that were replicated four times each. Each replicate (plot) was 1.5 × 1 m in size and the total area of 24 m² was maintained. Except for the control plots, each plot received approximately 3 kg of fresh straw. T0-No management (Control), T1- Mulching, T2-Burning, T3-In-situ incorporation as soil amendment. The soil testing was done at Biosystems Engineering Laboratory, Department of Biosystems Technology, Faculty of Technology, South Eastern University of Sri Lanka.

Soil sample collection

Two weeks after straw application, the soil samples at three random locations in each plot were collected at a depth of 10-15cm using a hand auger (8.2 cm diameter) and bulked to prepare composite soil samples. Before being submitted to further chemical and physical analysis, the soil from each plot was air-dried for two days and then sieved to remove debris and stones.

B. *Analysis of soil properties*

The preliminary soil physical properties such as colour and texture were characterized using Munsell colour chart (USA) and feel method. The moisture content of the soil was then determined using a gravimetric

method in a hot air oven at 105°C for 24 hours. The soil chemical properties such as pH, electrical conductivity were analyzed using benchtop pH meter (HP 9010) and multipara-meter (H1 9829) respectively. Prior to testing, instruments were calibrated according to manufacturer's instructions. Finally, the chromic acid wet oxidation method of Walkley and Black is used to measure soil organic carbon by oxidizing oxidizable organic carbon in the soil with potassium dichromate ($K_2Cr_2O_7$) solution in concentrated sulfuric acid.

Statistical analysis

Data collection were performed after fourteen days of straw applications to each treatments. All of the data was put into MS Excel, and descriptive statistics (mean, standard deviation) were calculated. ANOVA was also used to test the hypothesis and reveal significant differences between treatment means. Then, using SPSS, the means were compared using Tukey's post hoc range test at a 95% significant threshold (SPSS Statistics Version 22).

III. RESULTS AND DISCUSSION

A. Soil physical parameters

Soil moisture content:

The changes in soil moisture content with reference to different straw management options are shown in Figure 01. Mulching significantly increased the soil moisture content. This could be owing to the mulched materials' latent ability to shield the soil from evaporation and conserve moisture. Sarkar et al. [2] stated that crop residues improve soil porosity and water holding capacity. Further, burning (T2) non-significantly decreased the soil moisture content. This could be due to the

decrease in soil organic matter content, as organic matter is the primary component that holds soil water. On the other hand, straw incorporation (T3) also increased the soil moisture content but it was significant. This is in line with Chivenge et al. [3] who also found the rice straw incorporation increased soil water and nutrient holding capacity.

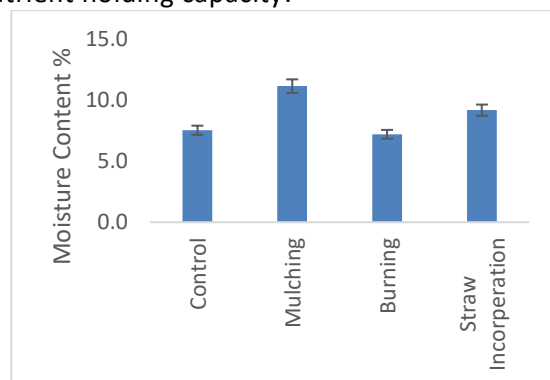


Figure 1. Soil moisture content in different straw management options after 14 days of application

B. Soil chemical properties

Soil pH

The mean values for pH in soils subjected to different straw management options are shown in Figure 2. Mulching and burning elevated soil pH considerably ($p < 0.05$) while in-situ incorporation as amendment showed more or less similar as well as lesser pH value to the un-managed (T0) condition. Lower organic matter content and decomposition rate might be the reasons for increase in soil pH.

The use of straw mulch, according to Khan et al. [4] has resulted in a slight increase in pH level. The study's findings were comparable to those of Sarkar et al. [2] who found that mulching and burning both increased soil pH.

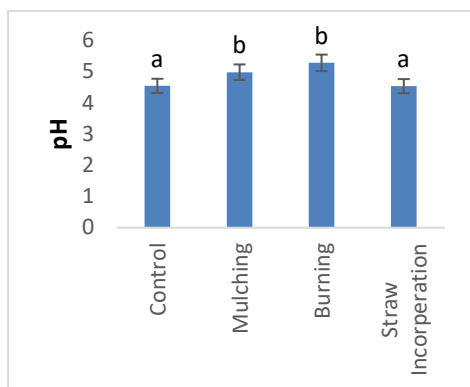


Figure 2. Soil pH in different straw management options after 14 days of application

Electrical conductivity

Electrical conductivity is an indirect indicator with a close relationship to several physical and chemical properties of soil.

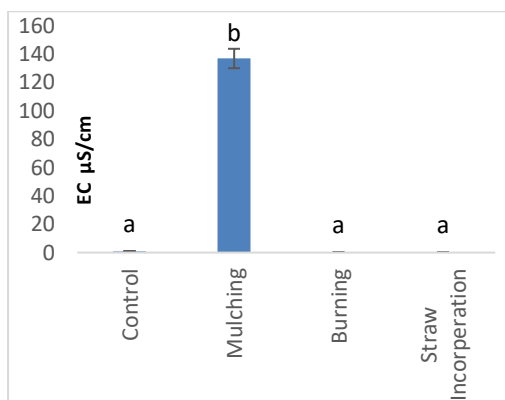


Figure 3. Soil EC content in different straw management options after 14 days of application

Figure 3 shows that straw mulched soil (T1) had higher electrical conductivity it was significant ($p < 0.05$) to other management options. Straw is a major source of organic fertilizer, which has an effect on the agro-ecosystem's and N cycles, as well as crop growth and yield. As mulching prevent water losses from the soil surface, dissolution of ions would be favoured inturn may increase the EC. Similarly, an increase in EC was observed by Aiome and

De Silva in coir dust mulched soil, where EC proportionally increased with the moisture content [12]. The straw layer improves the storage of salts in the top soil by acting as a barrier to the evaporation process [7]. Due to the destruction of soil capillarity in the straw layer, pore pressure water evaporation may be reduced, resulting in a rise in salts dissolved in the ground water and an increase in electrical conductivity [8].

Paddy straw burning led to a continuous decrease in soil electrical conductivity, according to Chivenge et al. [3]. Slow decomposition in the field also lowered the soil conductivity. Kumar and Kumar found that burning of straw decreased soil electrical due to lower decomposition [5].

Soil organic carbon

Soil organic carbon is the most major element of soil organic matter, as it helps to feed nutrients and enhances the soil's biological and physical qualities. The Figure 04 explains the changes in organic carbon content of soil where straw is managed in different manner.

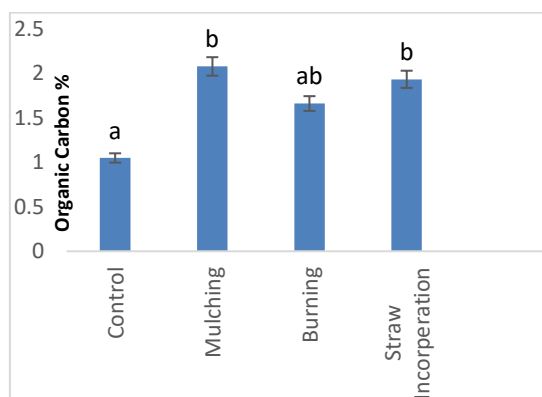


Figure 4. Soil organic carbon content in different straw management options after 14 days of application

It shows that straw management options namely mulching (T1) and incorporation (T3) into soil as amendment were significantly different ($p < 0.05$) for organic carbon content than control (no management). Hence, higher organic carbon content was recorded in the straw mulched soil. Since, straw is being an organic mulch, decomposition of it with the time might increase the organic carbon in soil.

Zhao et al. [8] discovered an 8% increase in organic carbon in the straw mulched plots. Furthermore, Thuan and Long discovered that adding rice straw to the soil increased soil organic carbon, pH, and nutrient content more than adding ash from burned straw [11]. Following incorporation, a wide range of soil microorganisms colonize the byproducts, causing decomposition and resultant mineralization. As a result, the residues are broken down into simpler monomers, which are then physically, chemically, and biologically assimilated and converted into organic matter and organic carbon [9]. Thuan et al. [6] found that the addition of rice straw improved soil pH, soil organic carbon content and soil microstructure.

Burning straw, on the other hand, reduced the amount of organic carbon in the soil. This could be as a result of reduced soil living organism which are beneficial for the decomposition of organic matters.

1V. CONCLUSIONS AND RECOMMENDATIONS

Rice straw is the most abundant agricultural residue and is also a low-cost source of ruminant food. Rice residues can be managed in a number of ways. Every

management option has advantages and disadvantages for managing rice residues. The present study identifies the potentials to solve the problem of rice residue management, which clearly indicated that straw mulching significantly increased soil moisture content, pH, electrical conductivity in short term periods. Conversely, in some situations, straw incorporation may be the best option and this speculation and the findings has to be studied in details. Furthermore, the diversifying mulching and straw incorporation may exert greater efforts in soil properties, so combining both management options may yield directly improve soil properties. If rice residues are managed properly, farmers can achieve improvements in soil productivity, minimize the reliance on the detrimental inorganic fertilizers and hence lead to achieve sustainable rice production in the country.

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SCREENING OF RICE GERMPLASMS FOR SUPERIOR ROOT, SHOOT MORPHOLOGIES AND DRY MATTER PRODUCTIVITY UNDER ANAEROBIC CONDITIONS IN SRI LANKA

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Abstract

Improved rice morphological and dry mass are highly concerned traits in novel-plant breeding programmes. This will ensure rice varieties to withstand abiotic stresses and sustain grain production in Sri Lanka. Therefore, the present study was carried out at Rice Research Station, Labuduwa with the aims of screening rice germplasms including best performing local varieties in low-country wet zone of Sri Lanka and additional germplasms conferred by IRRI for improved root and shoot morphology. The results indicated significant differences for most of the shoot and root characteristic among tested rice germplasms ($p < 0.05$). The highest plant height was observed in IRDTN 7–36 (120 cm) and Bw-367, while the lowest was observed in IRDTN 7–56 (66.3 cm). AERON 10-26 and Bw-367 had highest number of tillers per plant 9.5 and 7.0 respectively, whilst AERON 10–5 had the lowest tiller number per plant (4.3). Increased number of panicles per plant

were denoted in AERON 10-26 and Bw-367, while the highest grains per plant were obtained from Bw-367 compared to other rice germplasms. The highest individual panicle dry weight per plant was obtained in Bw-367 (7.50 g) followed by AERON 10–33 (7.12 g) and IRDTN 7–36 (6.49 g) while the least panicle dry weight was obtained in AERON 10–5 (2.91 g). Considering the root length, width, volume and number of roots per plant, the IRDTN 7–56 had the increased root length (21.4 cm) followed by IRDTN 7–36 (21.0 cm), local rice variety Bw-367 denoted with significantly increased root width (5.6 cm), volume (71.6 cm³) and number of roots (113/plant) than the tested counterparts. Results suggest that the overall performances of variety Bw-367 were superior or equal to the newly identified exotric rice germplasms AERON 10-26, AERON 10–33 and IRDTN 7–36 by the IRRI.

Keywords: Anaerobic condition, Germplasms, *Oryza sativa* L. Root morphology

I. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the staple food crop for human population and widely cultivated in tropical and subtropical regions of the world [1]. Globally, it is in the second place of cereal production next to wheat [2]. Even though Sri Lanka presumed

to be self-sufficient in rice production, the demand for rice is estimated to be increased by more than 1.1% per year [3]. According to Suriyagoda *et al.* [4], 2.9% annual growth in rice production is needed to meet that demand. Several studies were conducted previously propose that it is possible to

meet the demand by improving the yield capacity through crop breeding programs [5].

In Sri Lanka About 73,226 ha of land area in Low Country Wet Zone (LCWZ) is used for rice cultivation during *Maha* season 2014/2015 [6]. However, it is reported that rice production in LCWZ is comparatively lower due to several abiotic constraints *viz*: salinity, iron toxicity, flooding and poor drainage conditions [7]. Since, LCWZ act as a buffer zone of rice production, it is of paramount importance to increase the unit land productivity of rice in the LCWZ in order to ensure the national food security. Bg-300, Bg-359, Bg-358, Bw-367, Bw-272 - 6b and Bw-364 are the most popular rice varieties cultivated in LCWZ [8].

Rice is cultivated in a wide range of agro-climatic zones in Sri Lanka [9] and its production is mainly depended on rainfall [10]. Specially, in dry and Intermediate zones of the country, the growth and yield of paddy are largely determined by the seasonal rainfall variability either positive or negative anomaly [11]. For instance, in year 2014, paddy production was reduced by 27% due the drought condition prevailed in the country [12]. According to IPCC, [13], the global temperature is to increased by 1 °C over the next 50 years, and hence drastic changes in rainfall pattern, drought, flooding are possible in the tropical region. Thereby, in this context, developing climate- change resilient rice varieties are essential to withstand such scenarios, and the local rice breeding programmes need to prioritize to address future rice demand.

Characterization of germplasms is crucial in providing information on multiple traits carried by each accession to utilize in crop breeding programs [14]. Morphological,

biochemical and molecular strategies can be used to characterize germplasms [15]. Though, rice varieties are usually recommended based on their good yield performance, there are other important characteristics that should be considered [16]. Hence, characterization based on multiple morphological traits is important to improve rice varieties for potential adaptation [17]. Rice plant contains of an adventitious root system, stem (culm), leaves, panicles and tillers. These root and shoot systems are necessary to absorb and translocate water and nutrients [18]. Further, the dry matter production capacity is determined by the ability of shoots through photosynthesis, enables to synthesise, accumulate and carbon-partitioning among roots and shoot, eventually determine the final biomass and the grain yield [19]. Thus determining the optimized shoot morphological traits combined with improved physiological characteristics is paramount for improving rice grain yield production. Moreover, root morphology and physiology are strongly related with the growth and development of above ground plant parts [20]. The root system architecture has a significant impact on crop yield and it is mainly accountable for the adaptability and responses to varied stress situations *via* intricate gene interections [21]. Improved root characteristics help farmers to grow more stress-tolerant crops with increased yield by improving the crop potential for soil exploration [22]. Poor root and shoot characteristics in rice plants lead to lodging and low yield. Hence understanding the shoot and root morphology of rice plants is important to develop novel rice varieties using crop breeding program. Therefore, the objective of this study was to screen the locally available and newly released rice germplasm by the international rice

research institute for their root and shoot morphology under anaerobic conditions in the low country wet zone, Sri Lanka.

II. MATERIALS AND METHODS

Study area

The experiment was conducted at Rice Research Station, Labuduwa (6° 04'N and 80° 22'E; altitude 17 m above sea level) situated at the low country wet zone (WL₃) agro-ecological region of Sri Lanka from October to February during the 2020/21 *Maha* season. It receives an average rainfall of 2500 mm – 3000 mm per annum and the average annual temperature is 27 °C. The major soil type found in this area is Bog and Half Bog soil.

Planting materials and land preparation

A field trial was established under anaerobic condition with a total of ten rice varieties including best performing three varieties in low country wet zone and seven germplasms which were developed for low moisture tolerance by IRRI, through international drought nurseries and aerobic nurseries (Table 1) [23].

Table 1. Selected rice varieties for this study

Local rice varieties	Rice Varieties from IRRI
Bw272-6B	IRDTN 7-36
Ld253	IRDTN 7-11
Bw367	IRDTN 7-56
	AERON 10-33
	AERON 10-5
	AERON 10-26
	AERON 9-3

Initially, land preparation was done using two-wheel tractor-mounted rotovator according to the guidelines of the Department of Agriculture, Sri Lanka. Selected field was divided into 3 blocks of 6.7 m × 7 m. Nursery beds were prepared and seeds were broadcasted on 11th November, 2020. After 4th week of broadcasting, transplanting was done on 9th December 2020 at spacings of 20 cm x20 cm. The experiment was laid out in Randomized Complete Block Design (RCBD).

Crop management practices

All management practices including fertilizer application were performed as per the DOA recommendations. Triple super phosphate (TSP) and ZnSO₄ were applied as basal dressing before seed sowing at the rate of 55 kg/ha and 5 kg/ha respectively. 1st, 2nd and 3rd top dressing was done 3, 5 and 7 weeks after planting using urea (25 kg/ha, 30 kg/ha and 25 kg/ha respectively) and MOP (35 kg/ha, 45 kg/ha, 30 kg/ha respectively). Finally, 8 weeks after planting, urea was added at the rate of 21 kg/ha. Solito chemical was applied as the weedicide on 18th November 2020 and weeding was done manually 40-60 days after seed sowing.

Data collection and analysis

At booting stage, plant height (cm), flag leaf length (cm) and width (cm), number of leaves per bush and tillers per plant were recorded from randomly choosen five plants per replicates. Similarly, at harvest, number of panicles per tiller and mean panicle length (cm) were recorded from five plants per replicates. Plant height was measured from the base to the tip of the highest leaf and the panicle length was measured from the base of collar to tip of panicle [24].

Replicated samples were harvested and oven dried at 80 °C for 72 hours to obtain stem (including stalk and leaf sheath), panicle dry weight and number of grains per panicle, filled, unfilled grain weight, 50 grain weight were recorded [4]. Subsequently, root traits including number of roots, root length (cm) and width (cm), root volume (cm³) were recorded [25]. Root oven dry weight were recorded from randomly selected five plants in each replicates. Root length was measured from the base of the root to the end of the root tip of single long root [26] and all the other data were measured based on the IRRI standard evaluation system of rice.

Data were analyzed using Statistical Package for Social Sciences (SPSS) and the analysis of variance (ANOVA) was used for the statistical comparison of the treatments. The Tukey's post-hoc test was done to identify the significant difference between treatment means at 0.05 probability levels.

III. RESULTS AND DISCUSSION

Shoot characteristics of rice varieties

Evaluated rice varieties showed significant variations in most of the shoot characteristics. Plant height (cm), leaf width (cm), number of tillers per plant and stem dry weight per plant (g) showed significant variations among the tested varieties ($p < 0.05$) while leaf length (cm) and number of leaves per plant did not show any significant variations ($p > 0.05$) (Table 1). The highest plant height ($p = 0.006$) was observed in IRDTN 7–36 (120 cm) followed by Bw 367 (118.6 cm), AERON 10–5 (116.8 cm) and AERON 10–33 (110.2 cm) while the lowest was observed in IRDTN 7 – 56 (66.3 cm). Bw 367 recorded the highest leaf width

($p = 0.002$) (1.7 cm) while Ld 253 and AERON 10 – 5 recorded the lowest (1.1 cm). AERON 10–26 had highest number of tillers per plant (9.5/plant), ($p = 0.04$) whilst AERON 10–5 had the lowest number of tillers per plant (4.3/plant). Stem dry weight was significantly higher ($p = 0.04$) in AERON 10–26 and in BW 272–6B (11.2 g/plant) than the other tested rice varieties. In the present study, AERON-10-26 had the highest number of tillers per plant (9.5) followed by Ld-253 and BW-367 rice varieties

Plant height is an important trait related to the plant status and yield potential [14]. In a previous research conducted to study the morphological variation in selected rice germplasms of Sri Lanka, Suriyagoda *et al.* [4] obtained plant height ranging from 83.3 cm to 96.5 cm and stem dry weight ranging from 9.4 g/plant to 15.8 g/plant for Bg rice line varieties at the time of harvesting. Ld356 had 78.3 cm of plant height and 10 g/plant stem dry weight while for At303, the plant height was 86.8 cm and the stem dry weight was 13.5 g/plant. Meanwhile, Yang *et al.* [27] recorded 79.3 cm of plant height for At353 and 92.1 cm for Bg 352. In another study, Kakade *et al.* [26] stated that the number of leaves of 49 rice varieties in India ranged from 2 to 31. Pradheeban *et al.* [28] recorded 30.7 cm, 29.4 cm and 24.8 cm flag leaf length for Bg304, Bg403 and Bg357 rice varieties. Thus, the above studies show a diverse range of shoot characters are in line with the present experimental results. Local varieties performed well in terms of their morphological and dry matter production same as the IRRI varieties.

According to Kakade *et al.* [26], tillering is primarily focused that can help in improving rice yield potential. Productive tillering has a substantial impact on number of panicle in a unit area, hence it is crucial in determining

eventual grain production. A few tillers limit grain yield while excessive number of tillers can lead to high tiller abortion, small panicle size, poor grain filling and reduced grain yield [29]. Sivaneson *et al.* [24] obtained 14.29 number of tillers per hill and 5.62 (t/ha) of yield for Bw 361 rice variety while Suriyagoda *et al.* [4] obtained 15-29 tillers per hill for Bg rice lines and 15 for Ld356.

Table 2. Average performance of shoot characteristics of ten rice varieties under anaerobic field conditions

Varieties	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaves/plant	Number of tillers/plant	Stem dry weight/plant (g)
AERON 10 - 5	116.8±1.3 ^b	27.4±0.0 ^a	1.1±0.1 ^a	14.5±2.4 ^a	4.3±0.4 ^a	7.15±1.38 ^{ab}
AERON 10 -26	97.0±1.2 ^{ab}	24.9±0.2 ^a	1.3±0.1 ^a	18.3±2.1 ^a	9.5±1.4 ^b	11.55±2.82 ^b
AERON 10 - 33	110.2±1.6 ^{ab}	22.7±9.0 ^a	1.3±0.1 ^a	11.0±1.1 ^a	5.3±0.7 ^{ab}	6.60±0.87 ^a
AERON 9 - 3	100.3±0.0 ^{ab}	26.1±1.1 ^a	1.3±0.1 ^a	20.2±1.4 ^a	5.8±0.3 ^{ab}	6.68±0.28 ^a
IRDTN 7 - 56	66.3±27.5 ^a	22.7±3.0 ^a	1.4±0.1 ^{ab}	17.2±1.3 ^a	7.7±0.9 ^{ab}	6.42±0.05 ^a
IRDTN 7 - 36	120.0±1.9 ^b	33.9±0.7 ^a	1.4±0.1 ^{ab}	13.7±2.9 ^a	5.1±0.3 ^{ab}	7.33±0.00 ^{ab}
IRDTN 7 - 11	99.2±4.9 ^{ab}	29.8±1.0 ^a	1.4±0.1 ^{ab}	18.3±5.2 ^a	7.5±2.4 ^{ab}	7.10±0.08 ^{ab}
Bw 367	118.6±3.1 ^b	30.2±2.0 ^a	1.7±0.1 ^b	21.0±3.5 ^a	7.0±0.4 ^{ab}	7.02±0.11 ^{ab}
Bw 272 6B	105.6±1.9 ^{ab}	35.6±0.4 ^a	1.3±0.1 ^a	15.5±1.4 ^a	6.5±0.1 ^{ab}	11.20±1.46 ^b
Ld 253	78.3±0.4 ^{ab}	26.2±0.9 ^a	1.1±0.0 ^a	16.8±1.3 ^a	8.0±0.2 ^{ab}	8.15±1.09 ^{ab}
Mean	101.21	27.95	1.31	16.65	6.65	7.92
P value	0.006	0.102	0.002	0.249	0.042	0.040
CV (%)	21.01	22.13	15.95	28.43	31.25	31.46

The values are means of replicates ± standard error (SE); Within a column, means followed by the same letter are not significantly different by the Tukey's multiple range test at $p=0.05$. C.V = Coefficient of Variance

Panicles and grains characteristics of rice varieties

Number of panicles per plant, number of grains per panicle, panicle length (cm) and number of grains per plant were significantly differed among tested rice varieties ($p<0.05$), (Table 3). The newly improved germplasm AERON 10-26 had displayed the highest number of panicles per plant (9.2) followed by Bw-367, while IRDTN 7–36 had the lowest (4.8). concurrently, the highest number of grains per panicle was obtained in Bw 367 (177.3) followed by IRDTN 7–36 (119.0), Ld 253 (107.66) and AERON 10–33 (106.3) whilst the least number was denoted in IRDTN 7–11 (69.8). IRDTN 7–36 had the highest mean panicle length (24.0 cm) followed by Bw 367 and Bw 272 6B which both had 22.5 cm. The lowest mean panicle length was recorded in AERON 9–3 (18.4 cm). Bw 367 recorded the

highest number of grains per plant (980.0) while IRDTN 7–11 recorded the lowest (371.1).

In a previous study, Bw-361 rice variety recorded 14.23 number of panicles per hill and 20.17 cm of panicle length [24]. Suriyagoda *et al.* [4] obtained 112 – 264 total number of grains per panicle in Bg rice line varieties and 156 for both At303 and Ld356. Meanwhile, Peng *et al.* [30] obtained 343 grains per panicle in Bw451 and Bw363. Based on this analysis locally best performing varieties showed improved performances in terms of panicles and grains characteristics among ten rice varieties tested under anaerobic field conditions. Among the newly introduced varieties, AERON 10-26, AERON 10–33 and IRDTN 7–36 performed well.

Table 3. Average performances of panicles and grains characteristics in ten rice varieties under anaerobic field conditions

Varieties	Number of panicles/plants	Number of grains/panicles	Mean panicle length (cm)	Number of grains/plants
AERON 10 - 5	5.7±0.4 ^{ab}	73.2±13.2 ^{ab}	21.2±0.5 ^{bcd}	429.7±102.7 ^{ab}
AERON 10 -26	9.2±1.6 ^b	86.7±1.9 ^{abcd}	21.9±0.1 ^{cd}	803.7±159.5 ^{bc}
AERON 10 - 33	5.2±0.6 ^a	106.3±2.3 ^{bcd}	21.3±0.4 ^{bcd}	544.5±59.6 ^{ab}
AERON 9 - 3	5.0±0.4 ^a	77.8±6.4 ^{abc}	18.4±0.4 ^a	396.2±62.1 ^{ab}
IRDTN 7 - 56	5.3±0.4 ^a	74.8±2.0 ^{abc}	21.3±0.1 ^{bcd}	401.2±39.7 ^{ab}
IRDTN 7 - 36	4.8±0.1 ^a	119.0±5.9 ^d	24.0±0.4 ^e	576.5±40.5 ^{ab}
IRDTN 7 - 11	5.2±1.3 ^a	69.8±2.7 ^a	19.6±0.1 ^{ab}	371.1±101.6 ^a
Bw 367	6.2±0.1 ^{ab}	177.3±0.2 ^e	22.5±0.5 ^{de}	980.0±15.8 ^c
Bw 272 6B	5.7±0.2 ^{ab}	105.3±11.9 ^{bcd}	22.5±0.3 ^{de}	603.6±87.9 ^{ab}
Ld 253	5.5±0.3 ^a	107.7±6.2 ^{cd}	20.7±0.3 ^{bc}	597.5±64.9 ^{ab}
Mean	5.76	99.79	21.35	581.70
P value	0.020	<0.001	<0.001	<0.001
CV (%)	27.75	32.71	7.48	42.32

The values are means of replicates ± standard error (SE); Within a column, means followed by the same letter are not significantly different by the Tukey's multiple range test at $p=0.05$. C.V = Coefficient of Variance

Table 4. Average performances of yield characteristics of ten rice varieties under anaerobic field conditions

Varieties	Individual panicle dry weight (g)	Filled grain weight/panicle (g)	Unfilled grain weight/panicle (g)	50 grains weight (g)
AERON 10 - 5	0.52±0.01 ^a	3.93±0.88 ^a	0.59±0.08 ^a	0.48±0.01 ^a
AERON 10 -26	0.53±0.06 ^a	6.53±2.14 ^a	1.80±0.36 ^{bc}	0.38±0.01 ^a
AERON 10 - 33	1.43±0.13 ^b	5.55±1.03 ^a	1.34±0.14 ^{ab}	0.50±0.02 ^a
AERON 9 - 3	0.72±0.01 ^a	4.41±1.59 ^a	0.96±0.28 ^{ab}	0.50±0.02 ^a
IRDTN 7 - 56	0.70±0.10 ^a	5.46±0.40 ^a	1.46±0.30 ^{ab}	0.42±0.01 ^a
IRDTN 7 - 36	1.35±0.01 ^b	5.97±0.73 ^a	1.10±0.10 ^{ab}	0.55±0.03 ^a
IRDTN 7 - 11	0.68±0.05 ^a	7.95±2.09 ^a	1.00±0.17 ^{ab}	0.48±0.01 ^a
Bw 367	1.22±0.05 ^b	3.60±0.06 ^a	0.62±0.05 ^a	0.60±0.31 ^a
Bw 272 6B	0.67±0.02 ^a	6.93±1.27 ^a	2.84±0.21 ^c	0.28±0.12 ^a
Ld 253	0.55±0.09 ^a	5.92±0.64 ^a	0.80±0.15 ^{ab}	0.45±0.14 ^a
Mean	0.83	5.62	1.25	0.48
P value	<0.001	0.375	<0.001	0.280
CV	42.38	39.88	57.40	41.32

The values are means of replicates ± standard error (SE); Within a column, means followed by the same letter are not significantly different by the Tukey's multiple range test at $p=0.05$. C.V = Coefficient of Variance

Yield characteristics of rice varieties

Grain yield in rice is a complex trait and highly dependent on agronomic and reproductive characters [31]. Table 4 shows the average performance of yield characteristics of ten rice varieties under anaerobic field conditions. Individual panicle dry weight (g), filled and unfilled grain weight per panicle (g) were significantly differed among different rice varieties ($p < 0.05$) while significant variations weren't observed in filled grain weight per panicle (g), and 50 grains weight (g) ($p > 0.05$).

The highest individual panicle dry weight per plant was obtained in Bw 367 (7.50 g) followed by AERON 10-33 (7.12 g) and IRDTN 7-36 (6.49 g) while the least panicle dry weight was obtained in AERON 10-5 (2.91 g). Further, Bw 272- 6B had the highest value for unfilled grain weight per panicle (2.84 g) while AERON 10 – 5 had the lowest value (0.59 g).

In a previously conducted research, Bg line, At303 and Ld356 rice varieties had the number of filled grain numbers per panicle in the range of 85-227, 142 and 139 respectively. Moreover, the number of unfilled grain numbers per panicle were obtained as 5-41, 15 and 16 respectively [4]. Illangakoon *et al.* [32] obtained the values of 1.68 g and 1.31 g as the 100 grains weight for Bw 267-3 and Bw 272-6B rice varieties and Suriyagoda *et al.* [4] recorded 1.3 – 2.9g for Bg line, At303 and Ld356 rice varieties. Peng *et al.* [30] stated that Bw400, Bw452, Bw367, Bg400-1 and Bg 358 had more than 5 g of panicle weight. According to Wickramasinghe *et al.* [7], the average yield of Bw varieties were Bw- 367 (4.79 t/ha), Bw-372 (4.41 t/ha), Bw-267-3(4.18 t/ha) and Bw -361 (4.04 t/ha) in half bog soil. Based on present analysis, Bw-367, AERON

10-26, AERON 10-33 and IRDTN 7-36 performed better in terms of yield characteristics.

Root characteristics of rice varieties

Proper understanding of root morphology is important for developing new rice varieties that can sustain moisture stress in fields [33]. Table 5 shows the root characteristics tested among ten rice varieties in this study. Root length (cm), root width (cm), number of roots, root volume (cm³), root dry weight/plant (g) and root: shoot dry weight ratio showed significant variations among treatments ($p < 0.05$). IRDTN 7 – 56 had the highest root length (21.4 cm) followed by IRDTN 7-36 (21.0 cm) whilst Bw 367 had the lowest value (15.3 cm). The highest root width was obtained Bw 367 (5.6 cm) followed by IRDTN 7-36 (5.3 cm) while the lowest was obtained in Ld 253 (3.3 cm). The highest number of roots were observed in Ld 253 (113.7) and Bw 367 (113.3) followed by IRDTN 7-36 (92.2) while the lowest was observed in IRDTN 7-11 (52.8). Bw 367 had the highest root volume (71.66 cm³) followed by IRDTN 7-36 (62.16 cm³) in contrast Ld 253 had the lowest (20.16 cm³). The highest value for root dry weight per plant was recorded in AERON 10-26 (7.78 g) while the lowest was recorded in Bw 367 (2.42 g). IRDTN 7-11 had the highest root: shoot dry weight ratio (0.79) while Bw 367 had the lowest (0.3) (Figure 1).

Rice varieties with a long and thick root system, a high root to shoot weight ratio and root penetration capacity have been reported to contribute to yield under water stressed situation [34]. Roots having a small diameter and a long specific root length enhance the surface area of roots in contact with moisture, allowing more soil volume to be investigated for water [35]. Furthermore, the reduction in root diameter aids water

access and increases the productivity of plants under water stress [36]. According to Wasson *et al.* [37], deeper roots assist plants to access water and nutrient from deeper layers of soil column. Vejchasarn *et al.* [38] concluded that rice plants grown in waterlogged soils had more numbers of adventitious roots compared to those grown in drained soils or aerated solutions. The root-to-shoot ratio indicates the growth and development as well as the coordination of the underground and above ground crop parts. This ratio is also a good

predictor of a crop's ability to adjust to environmental conditions including water and nutrients [39].

In a previous study Sivaneson *et al.* [24] reports that 22.75 cm of root length for Bw 361 rice variety. In another study, Yang *et al.* [27] obtained 50.1 cm root length in At353 and 44.8 cm in Bg352 rice varieties. Kakade *et al.* [26] obtained 7 cm to 92 cm of root length and 0.013 cm³ – 14.08 cm³ of root volume for 49 different rice varieties in India.

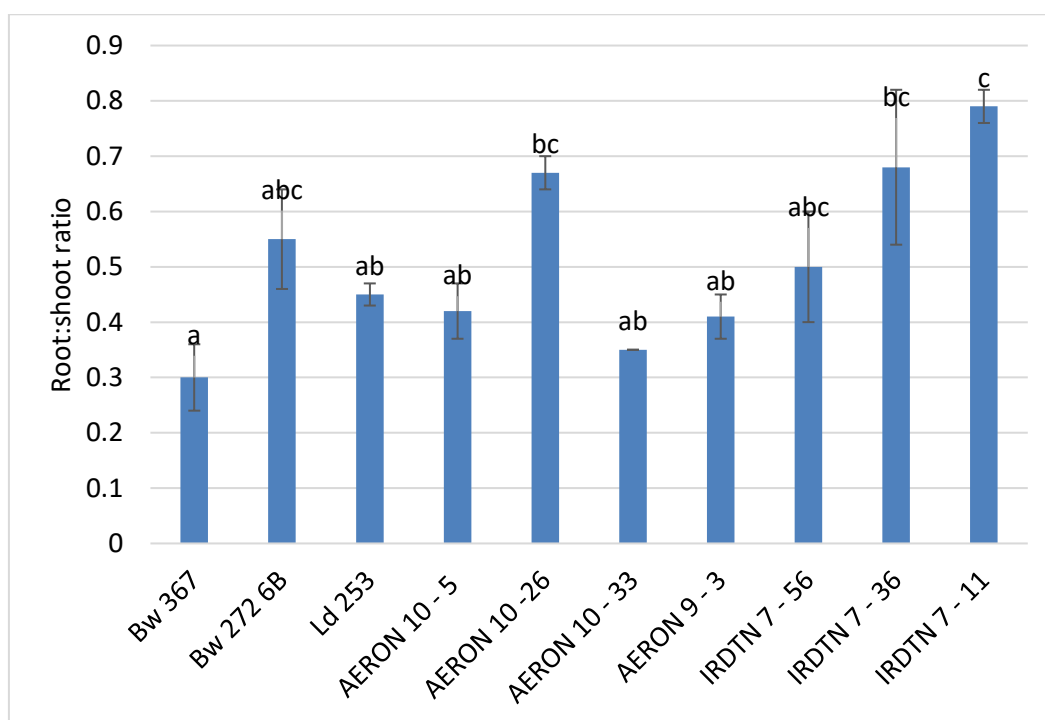


Figure 1. Average performance in root: shoot ratio of 10 rice varieties used in this study. Bars with same letters are not significantly different at Tukey's multiple range test at $p=0.05$.

Table 5. Average performance of root characteristics of ten rice varieties under anaerobic field conditions

Varieties	Root length (cm)	Root width (cm)	Root volume (cm ³)	Number of roots	Root dry weight/plant (g)
AERON 10-5	18.1±1.5 ^{abc}	3.6±0.1 ^a	24.5±0.5 ^{ab}	53.0±3.3 ^a	3.45±0.97 ^{bc}
AERON 10-26	16.5±0.7 ^a	4.1±0.2 ^{ab}	39.8±7.4 ^{abc}	63.5±8.9 ^a	7.78±1.55 ^b
AERON 10-33	16.1±1.0 ^a	4.5±0.3 ^{abc}	43.0±2.12 ^{bc}	72.3±5.6 ^{ab}	2.83±0.25 ^a
AERON 9-3	18.8±0.4 ^{abc}	4.4±0.1 ^{abc}	41.3±1.5 ^{abc}	73.2±5.3 ^{ab}	3.08±0.39 ^{bc}
IRDTN 7-56	21.4±1.1 ^c	4.5±0.3 ^{abc}	46.2±1.6 ^{bc}	73.7±2.5 ^{ab}	3.52±0.70 ^{bc}
IRDTN 7-36	21.0±0.2 ^{bc}	5.3±0.1 ^{bc}	62.2±3.2 ^{cd}	92.2±3.9 ^{bc}	5.92±1.16 ^{bc}
IRDTN 7-11	17.2±0.8 ^{abc}	3.7±0.4 ^a	37.5±5.8 ^{ab}	52.8±4.3 ^a	6.15±0.26 ^{bc}
Bw 367	15.3±0.4 ^a	5.6±0.5 ^c	71.6±8.6 ^d	113.3±4.6 ^c	2.42±0.45 ^a
Bw 272 6B	16.8±0.3 ^{ab}	3.7±0.3 ^a	40.5±4.1 ^{abc}	75.3±3.3 ^{ab}	6.92±1.84 ^{bc}
Ld 253	18.9±0.9 ^{abc}	3.3±0.0 ^a	20.2±0.8 ^a	113.7±4.2 ^c	3.93±0.59 ^{bc}
Mean	18.01	4.26	42.68	78.30	4.60
P value	0.001	<0.001	<0.001	<0.001	0.006
CV	12.88	19.53	37.79	28.32	50.25

The values are means of replicates ± standard error (SE); Within a column, means followed by the same letter are not significantly different by the Tukey's multiple range test at $p=0.05$. C.V = Coefficient of Variance

Similarly, In a previous research on comparison of shoot and root morphological development of selected rice lines under moisture conditions, Xu *et al.* [40] concluded that IRDTN 7–56 was the most drought tolerant rice line followed by the IRDTN 7-11 compared to Bg 300 line. Peng *et al.* [30] did a study to identify promising drought-tolerant rice lines based on the performance of agronomic traits under simulated drought conditions during reproductive and grain filling stages and concluded that the line IRDTN was the most promising entry while IRDTN 56, IRDTN 37 and IRDTN 22 also performed well under drought imposed conditions during the reproductive and grain filling stages. In another study, Wasana *et al.* [41] identified AERON 9-3, IRDTN 7-11 and IRBBN dhana as highly drought tolerant compared with BG 251. AERON 9-3 was identified as a cultivar with good yield as well as good Drought Tolerant Degree value. Moreover, studies also have shown that IRDTN lines have a high density of leaf trichomes and a well-distributed deep, thick root system enabling them to reduce water loss and to use water stored in deep soil layers during drought stress [42]. However, this study revealed that the local rice varieties have superior shoot, root and yield characteristics which are comparable to the elite IRRI varieties.

IV. CONCLUSIONS AND RECOMMENDATIONS

Clear understanding of root and shoot morphology and dry matter production capacities of rice germplasms is important for rice crop improvement programs to withstand predicted biotic and abiotic stresses and hence to sustain rice production. Based on the analysis it was found out that the local rice variety BW 367 performed superiority in terms of biomass and grain yield production and the performances are comparable to the elite newly introduced varieties AERON 10 -26, AERON 10 – 33 and IRDTN 7 – 36 when provided ideal experimental conditions.

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ASSESSING THE COMBINED EFFECT OF POULTRY MANURE AND INORGANIC FERTILIZERS ON GROWTH AND YIELD OF RICE: A CASE STUDY WITH A SRI LANKAN RICE VARIETY

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Abstract

Nutrients supplied exclusively through inorganic fertilizers is thought to enhance the yield of rice; however, the yields are not sustainable over time. Application of organic fertilizer alone cannot be a trustworthy solution for rice as it cannot provide the required amount of all nutrients rapidly due to its slow releasing nature of nutrients. The drawbacks associated with using only inorganic fertilizers or organic fertilizers could be overcome when they are used in integrated approaches as the combination improves plant growth and increases the yield and quality. Therefore, this study was conducted to assess the efficacy of combined application of poultry manure (2.5 t/ ha) with 100% inorganic fertilizer recommended by the Department of Agriculture, Sri Lanka compared to the same inorganic fertilizer recommendation alone. Results showed a clear response of irrigated rice (Variety Bg 379-2) to combine application of inorganic fertilizer and

organic manure during *Maha* season (October- February). From the present study, it was observed that application of poultry manure at 2.5 t/ ha along with 100 % recommended inorganic fertilizer increased yield components; panicles/ m², number of seeds/panicle, seed weight/ panicle (g) and 1000 seed weight (g) compared to the plants treated with inorganic fertilizer alone. Average yield increase due to poultry manure combination over control is 14 %. Therefore, combined application of poultry manure (2.5 t/ ha) and inorganic fertilizer can be suggested to the lowland paddy cultivations to improve yield and growth performances. However, with inorganic fertilizer restrictions, future research studies are important to find effective fertilizer combinations to sustain yield from improved varieties.

Keywords: Nutrient management, Organic agriculture, Organic fertilizer, Paddy

I. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for more than 3.5 billion people around the world, particularly in Asia, Latin America, and parts of Africa and one of the demanding meals of the rest of the world. Global rice production is estimated to be around 410 million tonnes with about 75%

of the production is claimed from irrigated rice production [1]. Rice is the single most important crop occupying 34% of the total cultivated area in Sri Lanka and on average 560,000 ha are cultivated during *Maha* (October- February) and 310,000 ha during *Yala* (March – September) making the average annual extent sown with rice to

about 870,000 ha in two cultivation seasons [2]. In Sri Lanka, rice is mainly cultivated as irrigated crop in all districts with current average paddy production is estimated as 4.5 t/ ha [3].

In order to assure the self-sufficiency of rice and to ensure food security globally, farmers must continue to reach higher productivity. The use of inorganic fertilizer for rice cultivation in Sri Lanka has been increasing specially for urea owing to the fertilizer subsidy scheme implemented by the government and due to the reduction in crop responses to the applied nitrogen levels [4]. Nutrients supplied exclusively through inorganic fertilizers is thought to enhance the yield of rice; however, the yields are not sustainable over time. Mainly, rice is supplied with nitrogen, phosphorous and potassium by inorganic fertilizers even though the plants require a complex set of macro and micro nutrients from the soil which is not consistently provided for rice in general. Thus, there has been a growing interest in the use of organic fertilizers for rice cultivations.

Different kinds of animal manure, poultry manure, cattle manure, rice straw, green manure such as *Gliricidia* have been evaluated and recommended for rice production [4,5,6]. However, application of organic fertilizer alone cannot be recommended for rice as it cannot provide the required amount of all nutrients and due to slow release of nutrients [6]. The drawbacks associated with using only inorganic fertilizers or organic fertilizers could be overcome when they are used in integrated approaches as the combination improves plant growth and increases the yield and quality [8]. Therefore, this study investigated the effect of integration of poultry manure with recommended inorganic fertilizer on growth and yield of

improved Sri Lankan paddy variety Bg 379-2.

II. MATERIALS AND METHODS

Experimental site and the rice variety

The experiment was conducted in rice fields of Kuliyaipitiya in the Kurungela district covering *Maha* season (October- February) of 2020/2021. The selected site was a conventional rice field applied with inorganic fertilizers consecutively for number of years. The soil of the experiment site belonged to the great soil group Low Humic Clay (LHG). The mean seasonal temperature was 28.5 °C with an average minimum temperature of 26 °C and maximum temperature of 31 °C.

Bg 379-2 white large grain variety was selected from the age group of 4- 4½ months which has been developed at Rice Research Institute, Bathalagoda, Sri Lanka.

Experimental designs and treatments

Two treatments were laid in adjacent farmer plots. Three containing 30 plant samples from each replicate was randomly selected to make a total of ninety plants per each treatment. The plot size was about 80 - 100m². For standard treatment (T1: control), inorganic fertilizers were applied as per the Department of Agriculture (Sri Lanka) recommendation supplying 90 kg (36.4 kg/ha) of urea, 25 kg (10.1 kg/ha) of Triple Super Phosphate (TSP) and 25 kg (10.1 kg/ha) of Muriate of Potash (MOP) per acre. For the integrated treatment (T2), poultry manure was applied at 2.5 t/ha just before harrowing and inorganic fertilizer was applied similar to the standard treatment (T1). For both T1 and T2, inorganic fertilizer was split-applied: For basal dressing, urea, TSP and MOP were applied at 5, 25 and 15 kg/acre respectively.

The 1st top dressing (urea only) was applied 14 days after seed sowing at the rate of 35 kg/ acre. As the 2nd top dressing, urea and MOP were applied at 50 and 25 kg/acre respectively splitting in to two applications.

Crop management

Commercial seed paddy of Bg 379-2 was purchased from Department of Agriculture and seeds were pre-germinated by alternate soaking and drying cycles for three days. Sprouted seeds were broadcasted by an experienced farmer on to the wet puddled and levelled fields.

Herbicide Pretilachlor 300 g/L EC was sprayed on the day of seed sowing to manage weeds. Fenobucarb 500 g/L EC was applied for brown plant hopper around two months after sowing.

Measurements and data collection

After ten days of seed sowing, plant height was measured, and number of leaves were counted. At the time of harvesting (115 DAP), the reproductive traits and yield components were measured; the number of panicles per m², primary branches per panicle, seeds per panicle, total number of tillers per plant, number of productive tillers per plant, 1000 seeds weight, panicle length and grain yield were recorded. Harvest index (HI= grain yield/ above ground dry weight) was calculated for both the treatments. Plant height, root length, shoot and root dry weights were taken as vegetative parameters at the time of harvesting.

Data analysis

Data were analysed in SPSS statistical software (Version 23) using One-way ANOVA at 95% confidence level.

productive tillers per plant ($p < 0.05$, Table 1).

III. RESULTS AND DISCUSSION

A. Effects of fertilizer type on initial growth of seedlings (at ten days after sowing)

The effect of fertilizers was significant on the height of seedlings and number of leaves at ten days after sowing ($p < 0.01$). The plants treated with both inorganic fertilizer and poultry manure (T2) had significantly taller plants with higher number of leaves than that of the plants treated only with inorganic fertilizer (T1) (Figure 1 a and b).

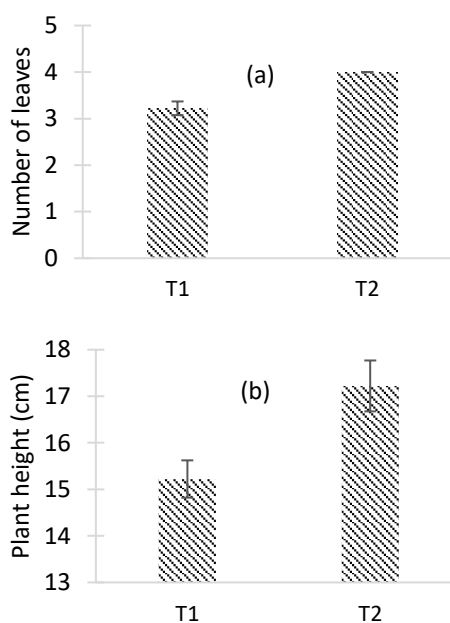


Figure 1. Effect of inorganic fertilizer and fertilizer-poultry manure combination on (a) height of the seedlings and (b) number of leaves at 10 days after sowing

B. Effects of fertilizer types on yield components and reproductive parameters

Effect of fertilizer type was significant on total number of tillers and number of

Table 8. Effect of fertilizer types on yield components and reproductive characteristics

Parameter	T1	T2	p value
No. tillers /plant	4.44 ^b ±0.18	12.33 ^a ±1.25	<0.01
No. productive tillers/plant	4.44 ^b ±0.18	11.11 ^a ±0.77	<0.01
Panicle length (cm)	22.94 ±0.06	23.00 ±0.00	0.33
No. seeds/panicle	104.56 ^b ±6.46	126.00 ^a ±6.24	0.03
No. primary branches/p anicle	10.67 ±0.47	11.89 ±0.61	0.13
No. panicles/m ²	266.67 ^b ±10.54	666.67 ^a ±46.30	<0.01
Seed weight/panicle (g)	1.94 ^b ±0.07	2.39 ^a ±0.17	0.02
1000 seed weight (g)	16.20 ^b ±0.36	23.14 ^a ±0.75	<0.01
Yield (t/ha)	4.47 ^b ±0.03	5.10 ^a ±0.06	<0.01
Harvest index	0.42 ±0.01	0.46 ±0.02	0.11

Table presents the mean values of three replicats. T1: Inorganic fertilizer only, T2: Combined application of inorganic fertilizer and poultry manure, The values denoted by different letters within columns are significantly different at 95% confidence level.

Average number of productive tillers for combined application of inorganic fertilizer and poultry manure was ~11 whereas single application of inorganic fertilizer produced ~4 productive panicles. Effect of fertilizer type was not significant on number of primary branches per panicle ($p > 0.05$, Table 1); however, number of seeds per panicle was significantly different between two treatments.

Table 9. Effect of fertilizer types on vegetative performance of rice at the time of harvesting

	Plant height (cm)	SDW (g/plant)	Root dry weight (g/plant)	Root length (cm)
T1	80.56 ^b ±0.90	12.11 ^b ±0.61	11.44 ^b ±0.38	10.78 ^b ±0.17
T2	87.00 ^a ±1.36	14.17 ^a ±0.58	27.67 ^a ±0.47	14.17 ^a ±0.26
p	<0.01	<0.01	<0.01	<0.01

Table presents the mean values of nine samples. T1: Combined application of inorganic fertilizer and poultry manure, T2: Inorganic fertilizer only.

The values denoted by different letters within columns are significantly different at 95% confidence level.

SDW: Shoot dry weight

Combined treatment produced panicles with larger number of seeds (126) compared to inorganic fertilizer treatment (105). The similar observation was made on seed weight per panicle, with the highest weight in combined treatment (2.39 g) and the lowest weight in inorganic fertilizer treatment (1.94 g). The number of panicles was higher in the plots treated with poultry manure and inorganic fertilizer (667/m²) when compared to the plots treated with inorganic fertilizer alone (267/m²) ($p < 0.01$, Table 1).

The 1000 seeds weight was higher in the rice plants treated with both inorganic fertilizer and poultry manure (23.14 g) when compared to the plants treated only with inorganic fertilizer (16.20 g). The highest paddy grain yields were observed in plots treated with poultry manure and inorganic fertilizer. Average yields of inorganic fertilizer and poultry manure treated plots were 5.10 t/ha while the yield for chemical fertilizer treated plots were 4.47 t/ha (Table 1).

The panicle length was not significantly different between the two treatments ($p > 0.05$, Table 1). The harvest index was not statistically different between two fertilizer treatments, due to the presence of higher percentage of unproductive tillers in integrated treatment ($p = 0.11$, Table 1).

C. Effect of fertilizer types on vegetative performance of the plant

The combined application of inorganic fertilizer and poultry manure produced significantly taller plants when compared to the plants treated only with inorganic fertilizer ($p < 0.01$, Table 2). The average height of the combined treatment was 87 cm whereas plants treated only with inorganic fertilizer were ~80 cm tall (Table 1).

The highest dry weight of both shoots (above ground) and roots and the root length were observed for the combined application of inorganic fertilizer and poultry manure ($p < 0.05$, Table 2).

The results showed a clear response of rice to combined application of inorganic fertilizer and poultry manure in terms of yield components and reproductive characteristics.

The percentage yield increases due to combine effect of poultry manure and inorganic fertilizer was 14% compared to the effect of inorganic fertilizer alone. Similar trend was observed in a study conducted [9]; application of organic manure (5 t/ha straw, 1 t/ha green manure and 4 t/ha cattle manure) along with inorganic fertilizer increased rice yield by 9% over inorganic fertilizer alone in *Maha* season. Also, another study demonstrated an increase of grain yield by 1.8% with incorporation of organic matter along with inorganic fertilizer [10]. Similar to the

present performance of combined treatment on yield components, higher number of productive tillers per hill and panicles/ m² were observed in plots treated with 1.5 t/ha organic fertilizer with inorganic fertilizer than plots treated with inorganic fertilizer alone [10]. The harvest index, number of tillers per hill, panicle length and number of spikelet per panicle are the most important characters that directly contribute to yield [11]. Even though combined treatment of poultry manure and inorganic fertilizers did not significantly increase panicle length and number of primary branches per panicle, higher number of seeds per panicle and higher number of productive tillers may have increased the yield compared to the plants of inorganic fertilizer treatment.

Accordingly, it is obvious that inorganic fertilizer alone would not be 100% effective in increasing yield and its components even though inorganic fertilizers such as urea, MOP and TSP supply nutrients (N, P and K) which are readily soluble in soil solution and thereby instantly available to plants to take up. However, organic manures have additional supply of micronutrients where microorganisms make the nutrients available gradually throughout the season. When there is no added organic manure to the paddy fields but are applied with inorganic fertilizer alone in the long run, organic C and N levels of the paddy soils may decline significantly [12]. However, according to a previous study, the level of soil organic matter was below the critical level of 3% even with of organic manure application for 11 years [13]. This could be explained by the slow accumulation of organic matter in the rice growing soils [9]. Thus, application of organic fertilizer alone has also not been effective in increasing yield of rice (Bg 352) compared to 100% inorganic fertilizer [5].

Integrated effect of farm yard manure and green manure with inorganic fertilizer demonstrated increased N uptake in rice indicating better utilization of applied nutrients under the combined application of inorganic and organic fertilizer sources of plant nutrients [14]. Similarly, in the present study with Sri Lankan rice variety Bg 379-2, commercial farmer plots have demonstrated the integrated effect of recommended dose of NPK fertilizer along with poultry manure can increase the yield and yield components of rice in irrigated paddy cultivation.

IV. CONCLUSIONS AND RECOMMENDATIONS

Results showed a clear response of irrigated rice (Variety Bg 379-2) to combine application of inorganic fertilizer and poultry manure during *Maha* season (October- February). From the present study, it was observed that application of poultry manure at 2.5 t/ha along with 100 % recommended inorganic fertilizer increased yield components; panicles/m², number of seeds/panicle, seed weight/panicle (g) and 1000 seed weight (g) compared to use of inorganic fertilizer alone. Average yield increase due to combination of poultry manure over control is 14 %. Therefore, combined application of poultry manure (2.5 t/ha) and inorganic fertilizer can be suggested to the lowland paddy cultivation to improve the growth and yield performances. However, with inorganic fertilizer restrictions, future research studies are important to find effective fertilizer combinations to sustain yield from improved varieties.

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FOCUS AREA
Health

OBESITY RELATED GENES AND THE RISK OF DEVELOPING PREECLAMPSIA: REVIEW FROM DISTINCT STUDIES

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Abstract

Obesity is a major public health and economic concern of global significance. Prevalence of overweight/obesity has continued to increase among women of reproductive age and predispose to various pregnancy related complications, such as preeclampsia. Preeclampsia is characterized by new onset of hypertension and proteinuria after 20 weeks of pregnancy. Although, it is widely known that obesity is a risk factor for preeclampsia, the mechanism behind obesity and preeclampsia is undetermined. Recent studies denote the association between obesity-related genes and preeclampsia with inconsistent results. The aim of this review was to analyse the association of obesity-related genes—ADIPOQ, FTO, LEP, LEPR, INSIG2, MC4R, PCSK1 and PPARG with preeclampsia.

Published literature that assessed the association between obesity-related gene polymorphisms and preeclampsia from PubMed/SCOPUS/Google Scholar/EMBASE from January 2000 to January 2020, was retrieved using the related keywords.

Four polymorphisms related to preeclampsia were found in ADIPOQ gene - rs17300539 (G>A), rs266729 (C>G), rs2241766 (T>G) and rs1501299 (G>T) and few studies reported CG genotype of the rs266729 was associated with preeclampsia. The most common polymorphism of FTO gene-rs9939609 was screened only in Finnish population and found no association with preeclampsia.

Further, many studies confirms that the LEP gene (rs7799039) polymorphism (G/A) and LEPR gene (rs1137101/rs1137100) polymorphisms (A/G) are significantly associated with preeclampsia. A few studies reported PPARG gene polymorphism may be a risk factor for the development of preeclampsia. It is noteworthy, that no studies have been carried out on obesity genes -INSIG2, MC4R, PCSK1 and their association with preeclampsia.

This review confirmed the significant association of LEPR gene polymorphisms with increased risk of preeclampsia. Further studies are required to verify the association of other obesity-related genes—ADIPOQ, FTO, INSIG2, MC4R, PCSK1 and PPARG with preeclampsia. Identifying the target genes and their mechanism will facilitate new insight into therapeutic discoveries.

Keywords: Preeclampsia, Polymorphisms, Obesity genes

I. INTRODUCTION

Obesity is a major epidemic in many countries around the world. According to the report by WHO, worldwide obesity has nearly tripled since 1975 [1]. In Sri Lanka, demographic and Health Survey (DHS) in 2000 and 2006/7 found 24% and 31.2% of women were overweight respectively [2,3]. Obesity leads to several complications during pregnancy, including fetal overgrowth, fetal malformations, spontaneous miscarriage, gestational diabetes, thromboembolic complications, stillbirth, preterm deliveries, caesarean section, and hypertensive complications [4]. Unhealthy lifestyle in the modern era may result in overweight and obesity in women of reproductive age and could predispose to various pregnancy related complications, such as preeclampsia [5].

Preeclampsia is a pregnancy specific disorder that appears after 20 weeks of pregnancy, and can be characterized by hypertension, proteinuria, edema of extremities, persistent severe headaches, visual disturbances, sudden onset of swelling of hands and feet, and hyperreflexia, etc. It affects coagulation, renal, respiratory, and central nervous system and can have detrimental consequences on the placenta and the baby [6]. The exact pathogenesis remains unclear, but both genetic and environmental factors may play a major role in the development of preeclampsia.

Obesity is considered as one of the risk factor for preeclampsia and there are many common mechanisms that link obesity with a higher risk of developing preeclampsia [7]. Most importantly, the occurrence of preeclampsia has increased over the past years and obesity prevalence has also been on the rise [8].

Preeclampsia has remained a significant public health threat in both developed and developing countries contributing to maternal and perinatal morbidity and mortality globally [9]. Sri Lanka saw a decline from 6.3 per 1000 maternal deaths in 1948 to 0.8 per 1000 maternal deaths in 1977 in the maternal mortality rate (MMR) [10]. During 1990 to 2010, the MMR further reduced from 85 per 100,000 maternal deaths to 35 per 100,000 maternal deaths [11]. As Sri Lanka now targets towards a single digit in the maternal mortality rate, there is an urgent need to reduce our maternal deaths further [11]. Therefore, there is a necessity to screen the risk factors of preeclampsia during the antenatal period.

Most importantly, effective treatment for women with severe eclampsia is delivery of the baby by caesarean. Therefore, efforts to prevent and reduce morbidity and mortality due to this condition can help address the profound inequities in maternal and perinatal health globally. Identifying the risk

of developing preeclampsia at the early stage may prevent the preeclampsia mortality. It is noteworthy that, genetic cause can be identified at the very early stage and early detection of the disorder is a crucial element in the prevention of preeclamptic mortality [12].

Studies have shown that obesity increases the risk of preeclampsia about 3-fold, and in developed countries is the leading attributable risk for the disorder [13]. The relationship between preeclampsia and obesity has been greatly studied due to the high prevalence of preeclampsia in pregnant women. However, only a few studies have explored the obesity genes and their association with preeclampsia [14-22]. Hence, it is necessary to identify the possible obesity related genes and their association with preeclampsia.

While numerous epidemiological studies have demonstrated that obesity increases the risk of preeclampsia, the mechanisms have yet to be fully elucidated. Understanding how obesity increases the risk of preeclampsia is important for several reasons. Since the obvious cure for obesity, weight loss, is not an appropriate strategy during pregnancy and minimally successful in the modern era due to the practice of unhealthy eating habits. Since obesity and overweight are major problems that lead to significant health and social difficulties around the world, in

contrast to other risk factors for preeclampsia, it would seem that mechanism involving obesity to contribute to preeclampsia are more likely to be relevant to the general population. Most importantly, identifying the target genes that have influence on obesity and preeclampsia, might facilitate the development of therapeutic drugs that can react with or modulate the targets and thus, eventually reduce the risk of preeclampsia in pregnant women. Therefore, this review evaluated the role of different obesity genes and their association with preeclampsia.

This review aimed to analyse the selected obesity related genes and their association with preeclampsia and evaluate the current evidence of the role of selected obesity related genes such as ADIPOQ, FTO, LEP, LEPR, INSIG2, MC4R, PCSK1 and PPARG in the pathogenesis of the development of preeclampsia.

II. MATERIALS AND METHODS

Studies that assessed the association between obesity related genes and preeclampsia from January 2000 to January 2020 were searched in PubMed/SCOPUS/GoogleScholar/EMBASE. All studies that assessed the association between obesity related genetic variants and preeclampsia were included in the review. We used the following search terms:

“preeclampsia”, “obesity” and “genes”.

A. Inclusion and exclusion criteria

Inclusion criteria, included: 1) the studies with complete text in databases, 2) case-control, cross sectional and cohort studies with appropriate design, 3) studies by human samples, 4) study population including pregnant women.

Exclusion criteria included: 1) inappropriate design of study and 2) genetic studies on preeclampsia other than obesity related genes.

B. Data extraction

The qualified studies which possessed the inclusion criteria were investigated and the following information's were extracted: 1) characteristics of the study (year of publication); 2) the number of participants (cases and controls), 3) Study population (ethnicity) and 4) gene and polymorphisms studied 5) association of obesity related genes with preeclampsia (results).

III. RESULTS AND DISCUSSIONS

The results of association of obesity related genes and preeclampsia were listed in Table 1 (Annexure 1).

In this review we found only LEP gene (rs1137101/rs1137100) polymorphisms (A/G) were found to be significantly associated with preeclampsia in different studies across various ethnicity. However, only few studies have been analysed the association of preeclampsia with

LEP gene (rs7799039) polymorphism. A study by Machado et al., identified 4 single nucleotide polymorphisms (SNPs) in ADIPOQ genes in related to preeclampsia [rs17300539 (G>A), rs266729 (C>G), rs2241766 (T>G) and rs1501299 (G>T)], it is noteworthy among the 4 SNPs of ADIPOQ genes only rs266729 CG genotype showed modest association with preeclampsia [21]. A recent study concluded PPARG gene SNPs rs10865710 and rs4684847 may be associated with the risk of developing preeclampsia in Chinese population [22]. Most importantly, no studies have been carried out on other obesity related genes such as INSIG2, MC4R, PCSK1 genes and their association with preeclampsia.

Increasing body mass index (BMI) is positively associated with an increased risk of preeclampsia. Women with a pre pregnancy BMI of 35 kg/m² or above have a 30% increased risk of developing preeclampsia [23]. It is noteworthy, that only 10% of obese women will develop preeclampsia during their pregnancy [24]. Therefore, it is important to examine the reason why some but not all obese women develop preeclampsia and the reason may be found by studying the genetic of obesity related genes and their association with the disease. Although, many different mechanisms have been proposed as explanations of the pathophysiology of preeclampsia, the association of

obesity genes and preeclampsia is not well defined.

We believe that focusing on the obesity related genes and their association with preeclampsia may shed fresh light on providing a more complete picture on the genetic basis of preeclampsia. Heritability can be studied by 4 methods - twin studies, family association studies, candidate gene studies and Genome Wide Association Studies (GWAS). In this review we have analysed the heritability based on the candidate gene studies in relation to the obesity genes and preeclampsia.

In this study we consistently found evidence strongly supporting the link between leptin and leptin receptor polymorphisms with preeclampsia. One of the weaknesses linked to leptin polymorphisms is many studies have determined the activity of leptin by using leptin hormonal assay instead of leptin gene (LEP) screening. This could be due to the fast and reliability of hormonal assay when compared to the genetic screening. Hence, the polymorphisms associated with LEP gene is not widely studied in many research that determined the association of leptin with preeclampsia. Leptin acts by binding to its receptor, LEPR. However, studies have confirmed that leptin receptor polymorphisms are associated with preeclampsia across different population and can act as a risk factor for the development of preeclampsia [14, 16]. A study by Poston concluded

that various processes can be induced by augmented leptin levels, for example increased sympathetic activity and mitochondrial superoxide synthesis, expansion of Th1 cells secreting pro-inflammatory cytokines, etc. All these mechanisms ultimately result in oxidative stress, endothelial and placental dysfunction, and leads to preeclampsia [24]. In addition, a gene microarray study proved that a high expression of the leptin gene is seen in pregnant women with preeclampsia [25]. Nevertheless, it is still debated whether raised leptin levels are a cause or a consequence of preeclampsia.

IV. CONCLUSION AND RECOMMENDATIONS

Many studies failed to prove any significant association between obesity related genetic variants and preeclampsia. This may be due to the clinical variety of the cases that were included in the studies. Some studies, for instance, included only women with severe preeclampsia. It is, however, also likely that there is a true lack of association between preeclampsia and these genetic variants. In addition, discrepancy of findings is also likely be due to variation in study design, sampling technique, genotyping method used and sample size along with demographic and genetic differences among the study population. To conclude, it can be assumed that among the obesity related genes which were reviewed

in this study, only leptin receptor gene polymorphisms may act as a risk factor for the development of preeclampsia across different ethnicity and these findings no doubt require validation by further larger scaled studies.

The aetiology of preeclampsia remains unknown and there is an urgent need for effective strategies for preventing preeclampsia. The identification of genetic variants associated with preeclampsia susceptibility can lead to novel biological insights and result in new targets for the prevention and treatment of preeclampsia. However, in order to prevent any bias, genetic association studies should preferably be performed using large scale studies. Furthermore, future studies should also focus on assessing the relevance of previously not studied obesity genes; for instance, INSIG2, MC4R, PCSK1 genes and their association with preeclampsia. Most importantly, reproduced genetic variants such as LEP and LEPR gene polymorphisms should be analysed in larger scale studies across different ethnicity. Alternatively, genome-wide association studies and next-generation sequencing methods should be used for identifying new susceptibility obesity related genes of preeclampsia. In addition, next-generation sequencing may identify rare causal variants that are associated with preeclampsia.

In summary, this review identified LEPR gene polymorphisms are associated with preeclampsia and the genetic variant of LEPR polymorphisms are likely to represent true associations. Further studies investigating the relative contribution of other obesity related genes variants and the mechanisms by which they affect the risk of developing preeclampsia are warranted.

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ABBREVIATION

ADIPOQ – adiponectin gene; FTO – fat mass and obesity-associated gene; LEP – leptin gene; LEPR – leptin receptor gene; INSIG2 - Insulin Induced Gene 2; MC4R - Melanocortin 4 receptor; PCSK1 - Proprotein Convertase Subtilisin/Kexin Type 1; PPAR γ - Peroxisome Proliferator Activated Receptor Gamma.

AN EXPLORATORY STUDY OF VETERINARY PROFESSIONALS' ATTITUDES AND PERCEPTION ON COMPANION ANIMAL EUTHANASIA IN SRI LANKA

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Abstract

Euthanasia is the act of deliberately ending an animal's life to relieve suffering. It is a controversial topic in Sri Lankan context due to cultural and religious beliefs. This study was designed to investigate Sri Lankan veterinarians' attitude towards euthanasia of companion animals. A total of 432 veterinarians participated in the study. Most veterinarians (51%) were companion animal practitioners who have 5-6 years clinical practice (68%). Most veterinarians (88%) usually perform 1–2 euthanasia per month, administering only an overdose of general anaesthetic (65.1%). Commonly used communication practices by veterinarians to explore the clients' expectations regarding euthanasia included, inquiring about client's emotional status (77.7%) and client's support network at home (41.3%). The support practices used in providing compassionate care to clients during euthanasia included, showing empathy/ sympathy (61.3%), suggesting options for consolation (buying/adopting new pets) (39.8%), providing comfort items (a bottle of water, tissues, places to sit) (31.2%) and ensuring the client has recovered/follow up care (7.5%). The participants' years of experience was positively and significantly (Minitab 19, 95% CI level) associated with veterinarians' use

of communication practices and support practices. For many veterinarians (75%), two conditions were necessary to justify euthanasia; "the animals should be incurable and suffering" and "the owners should request to euthanize." In the absence of either condition, the veterinarians were inclined to refuse euthanasia. If owners requested further treatment for animals with serious medical conditions, 80.9% showed clear disapproval. On average 97% showed clear disapproval of euthanizing healthy animals upon owners' request. These results indicate that owners' request takes precedence over the animals' condition for suffering animals, but not for healthy animals. This study revealed important facts regarding veterinarians' perception of euthanasia of companion animals. Many research questions still remain unanswered, such as owners' views of euthanasia and veterinarians' stress management. Future studies, should be directed at elucidating these issues and seek a better way of managing companion animals' terminal care.

Keywords: Euthanasia, Companion Animals, Veterinarians

I. INTRODUCTION

The escalating recognition of the inseparable bond which exists between humans and animals has widened and upgraded the standards of practice and teachings within the veterinary profession in Sri Lanka. Similar to many other healthcare professions, veterinary medicine offers a broader and more holistic approach to cure animals and help human health and wellbeing. In addition to looking after the wellbeing of their patients, veterinarians greatly incorporate considerations for the social, emotional and psychological wellbeing of its clients [1].

Euthanasia is the act of deliberately ending an animal's life to relieve suffering. However, euthanasia of companion animals is considered as one of the most stressful aspect of veterinary medicine. Veterinarians have to deal with both the suffering animal and the grieving client. The utmost role of companion animal veterinary medicine is extending a beneficial and enjoyable life to animal patients while preventing or reducing suffering. Veterinarians can actively grant "merciful relief" from irreversible pain or an incurable ailments, which makes the veterinarians different from the rest of the healthcare professionals [2].

The euthanasia of companion animals has become a controversial topic worldwide because of the way general public views this phenomenon. How an individual looks at euthanasia, therefore, can make a massive difference in the resulting experience of grief. Euthanasia often renders significant stress for both the client and the veterinarian and is often as emotionally challenging for the veterinarian as for their clients equally [3].

It has been found that, the process of deciding and administering euthanasia for companion causes tremendous level of stress and grief for many veterinarians and owners in Western countries like Canada and the United States [4]. If so, it can obviously be assumed that, the process would likely cause even more stress and grief for veterinarians and owners in Sri Lanka, where euthanasia tends to be viewed more negatively. In spite of that, as yet there is no consensus of opinion on euthanasia among Sri Lankan veterinarians. Even among veterinarians, there has been no public discussion of how to manage the process, and it is left to the discretion of each veterinarian's individual thoughts [5].

Nowadays, in Sri Lanka, while dogs and cats enjoy longevity, the number suffering from incurable diseases is also increasing. In such situations, owners and veterinarians usually have to consider whether they should continue treatment or terminate the life of the pet. According to author's experience, Sri Lankan pet owners show a significant reluctance to deliberately put an end to the life of companion animals and for many Sri Lankans euthanasia could be a difficult but inevitable option which in turn exerts a significant pressure to veterinarians as well. Therefore, it was envisaged to assess the background information of this phenomenon, due to the critical role played by the veterinarians in the treatment of companion animals, especially their input into end-of-life situations. The aim of this study is to investigate Sri Lankan veterinarians' attitude towards euthanasia of companion animals. It is undeniably important to seek how Sri Lankan veterinarians actually manage this stressful

process and clarify the ambiguities woven around the companion animal euthanasia.

II. MATERIALS AND METHODS

Questionnaire designing

An online questionnaire was designed using Google forms. The questionnaire was organized into four sections: (1) Demographic characteristics of the veterinarians, (2) Perceptions about different examples of euthanasia (3) Exploration of clients' experiences/expectations and providing emotional support and compassionate care to clients during companion animal euthanasia and (4) Moral criteria for choosing euthanasia. Participants were informed that the survey data would be used for academic purposes and that they would remain anonymous.

Questionnaire distribution

A target sample size (n=345) was calculated using a conservative approach (p=0.5) with 5% error and 95% confidence. Before the distribution, the questionnaire was pretested with a known convenient sample of veterinarians (n=15). The obtained feedback was used to revise questionnaire length, clarity of language, flow and completeness. Participants' responses in pretesting were not included in the study sample. The finalized questionnaire was administered through the online platforms. Eligible participants included all veterinarians in Sri Lanka.

Participant demographic information

The questionnaire contained demographic and social background questions such as gender, highest education level (BVSc, Masters, Doctorate), type of work (small animal practitioner, large animal practitioner, mixed practitioner), number of years in veterinary practice and the clinic's location (urban, suburban or rural). The present study focused on general questions euthanasia such as the number of euthanasia cases administered in a month, the usual place of performing euthanasia in the clinic, methods adopted, regrets felt after performing euthanasia, stress experienced in the process of euthanizing animals.

Perceptions about different examples of euthanasia

On a 5-point Likert scale (1=strongly disagree; 2=disagree; 3=neither agree nor disagree; 4=agree, and 5=strongly agree) participants were asked to express their degree of agreement to a set of questions which inquired about different examples of companion animal euthanasia. These examples included serious medical conditions that can no longer be managed medically and cause further suffering, serious and long-lasting behavioral problems such as aggressive behavior, as a justification for euthanasia, euthanasia in cases of a zoonotic disease that cannot be treated or medically managed, euthanasia as a population control measure (stray cats /dogs), and euthanasia is never justifiable, whatever the reason.

Providing emotional support and compassionate care to clients during companion animal euthanasia

As already mentioned, companion animal euthanasia is a stressful moment for both the client and the vet equally. Therefore, questions were included to assess the veterinarians' use of several communication and supportive practices to ease the stress of the client at an event of euthanasia. On a 5-point Likert scale (1=never; 2=rarely; 3=sometime; 4=very often; 5=always), participants were asked to report how often they inquired about a client's expectations and emotional experience during the euthanasia process [6].

Furthermore, on a 5-point Likert scale (1=strongly disagree; 2=disagree; 3=neither agree nor disagree; 4=agree, and 5=strongly agree), it was assessed, how important participants felt it was to inquire about the expectations of clients and their willingness to alter their euthanasia practices based on clients' expectations.

Emotional support of stressed clients

Participants were asked to rate how often on a 5-point Likert scale, (1=never, 2=rarely; 3=sometime; 4=very often; 5=always) they used different support practices in order to assist the client in such a stressful moment. These practices included, providing comfort items, showing sympathy/empathy, normalizing emotions, suggesting options for consolation/relieving stress of the client and participants' use of follow-up care such as phone calls.

Moral criteria for choosing euthanasia

The questions about moral criteria for choosing euthanasia were included in the questionnaire to assess how the veterinarians' moral values deal with the decision making in an example of euthanasia. The common question was "Do you think that it is better to euthanize animals or not in the following cases?" and this was followed by 10 circumstances describing different situations in terms of owners' request for euthanasia, animals' pathological condition, and owners' ability to pay (Table 1). Each item offered two response choices: "Should euthanize" or "Shouldn't euthanize". They were designed to discover whether veterinarians suggest euthanasia when owners request further treatment and to compare with the veterinarians' own judgments about choosing euthanasia.

Table 1. Question items on moral criteria for choosing euthanasia

	Question items about moral criteria for choosing euthanasia
01	When there is no hope of recovery and you cannot relieve their pain or suffering, even if you provide medical care to the animals, and the owners request to euthanize them
02	When there is no hope of recovery and you cannot relieve their pain or suffering, even if you provide medical care to the animals, but the owners request to continue treatment
03	When there is hope of recovery if you provide medical care to the animals, but it is predicted that the animals' quality of life will be greatly decreased after recovery
04	When there is no hope of recovery, even if you provide medical care to the animals, and the owners do not request to euthanize them, but the owners' quality of life has been greatly decreased
05	When there is hope of recovery if you provide medical care to the animals, but the owners

	request to euthanize them because they cannot pay for the treatment
06	When there is hope of recovery if you provide medical care to the animals, but the owners request to euthanize them because they cannot pay for the treatment
06	When there is hope of recovery if you provide medical care to the animals, and the owners request to continue treatment but cannot pay for the treatment
07	When the owners request to euthanize the animals because they cannot take them to a new address
08	When the owners request to euthanize puppies or kittens whelped by pets they keep
09	When neighbors request to euthanize puppies or kittens whelped by stray dogs or cats
10	When relatives or neighbors request to euthanize pets of owners who have been hospitalized or have died

Data Analysis

Completed questionnaire data were entered and reviewed in a database management program (Microsoft Excel Software, Version...), and then imported, analyzed and modelled using standard statistical software (Minitab 19). Descriptive statistics were generated including means and the associations were assessed using ordinal logistic regression method. (Significant at 95% Confidence Interval Level)

III. RESULTS AND DISCUSSION

A total of 432 veterinarians completed the questionnaire. Participants were primarily women (77%). Most participants (54%) were companion animal practitioners and employed in the clinics located in an urban area (63%) and used a separate examination room in the clinic to perform euthanasia (55%). Eighty-eight per cent of participants were employed in a clinic that usually

performs 0–2 euthanasia per month which performs euthanasia by administering only an overdose of general anesthetic (65.1%). Many participants (68.9%) discuss with their colleague/s before deciding on euthanasia and relatively a small portion (15%) of veterinarians regret after performing euthanasia. Most veterinarians (65.1%) administer only an overdose of general anesthetic when performing euthanasia.

Table 2. Participants' (n=368) descriptive statistics

Variable	Percentage
Sex	
Female	77%
Male	23%
Years of Clinical Practice	
1-5	22.7%
6-10	45.9%
11-15	20.6%
16-20	10.8%
Highest Level of Education	
Bachelor	86.2%
Masters	11.7%
Doctorate	1.1%
Clinic Type	
Companion animal	54%
Large animal	10.8%
Mixed	35.2%
Clinic Location	
Urban	62.9%
Sub-urban	32.6%
Rural	3.4%
Location of Euthanasia	
Separate Procedure Room	55%
Common Examination room	32.7%
Clients house	12.3%
Number of Euthanasia/Month	
0-2	88.9%
3-5	8.6%
>5	2.5%
Methods using for Euthanasia	
Administration of a sedative followed by a special euthanasia agent	13.3%

Administration of I/V Magnesium Sulphate	16.3%
Administration an overdose of general anesthetic only	65.1%
Administration of overdose of specific muscle	5.3%

Among the given examples of euthanasia, the higher percentages of veterinarians strongly agreed/agreed on euthanizing animals with serious conditions that can no longer be managed medically and cause further suffering (73.4%) and zoonotic disease that cannot be treated or medically managed (77.6%). Veterinarians have shown a comparatively clear disapproval for euthanizing companion animals due to their behavioral problems (77.6%). Highest disapproval rate (90.5%) was shown for euthanizing animals as population control measure. Around 16% of veterinarians strongly agreed/agreed that euthanasia is never justifiable whatever the reason.

A higher number of participants (91%) strongly agreed that it was important to assess the individual wishes of clients during the companion animal euthanasia process. The communication practices used by veterinarians to explore the clients' expectations, in the order of most commonly used to least commonly used were inquiring about clients' emotional status (77.7%), inquiring about client's support network at home (41.3%) and inquiring about clients' relationship with their companion animal (38.2%) and inquiring about the clients previous experience with euthanasia (23.2%) (Figure 1).

The highly employed three support practices that were reported to always be used in providing compassionate care to

Table 3. Degree of agreement for different examples of companion animal euthanasia

Example of Euthanasia	Strongly Agree/Agree	Neither agree nor disagree	Strongly Disagree/Disagree
Serious conditions that can no longer be managed medically and cause further suffering	73.4%	10.6%	16%
Serious and long-lasting behavioral problems such as aggressive behavior, as a justification for euthanasia	7.4%	14.9%	77.6%
Euthanasia in cases of a zoonotic disease that cannot be treated or medically managed	60.6%	26.6%	12.8%
Euthanasia as a population control measure (stray cats /dogs)	3.2%	6.4%	90.5%
Euthanasia is never justifiable whatever the reason	16%	25.5%	58.5

clients by participants included, showing empathy/ sympathy (61.3%), suggesting options for consolation (buying/adopting new pets) (39.8%), providing comfort items (a bottle of water, tissues, places to sit) (31.2%), however, usage of follow up care ensuring the client has recovered showed a comparatively lower percentage (7.5%) than the other practices (Figure 2).

As demonstrated in tables 4 and 5, the number of years in practice was positively and significantly associated with all communication practices except one practice (Inquiring about clients support network at home). Only two support

practices were showing significant association with the veterinarians' years in practices.

Ten question items were included in the questionnaire to discover veterinarians' moral criteria for choosing euthanasia (Table 1). For question item A, where owners request euthanasia for the incurable and suffering animals, 75.5% chose "should euthanize," while only 24.5% chose "shouldn't euthanize." Meanwhile, for the other nine question items, less than 20% chose " should euthanize," and over 90% chose "shouldn't euthanize

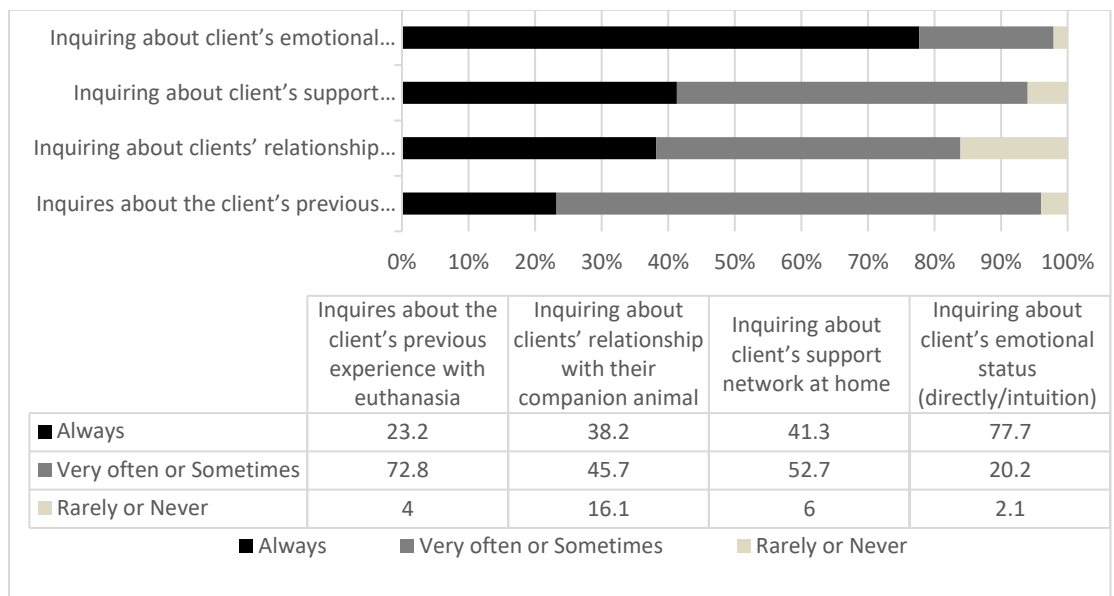


Figure 1. Exploring clients' experiences, expectations and emotions during companion animal euthanasia

Table 4. Results of ordinal logistic regression model examining the association between veterinarians' years in practice and the extent to which veterinarians' explore a client's experiences, expectations and emotions during companion animal euthanasia

	Use of communication practices to Explore clients' experiences, expectations and emotions	P value	Significance at 5%
01	Inquiring about client's emotional status(directly/intuition)	0.002	Significant
02	Inquiring about the clients relationship with their animal	0.033	Significant
03	Inquiring about clients previous experience with euthanasia	0.038	Significant
04	Inquiring about clients support network at home	0.553	Not Significant

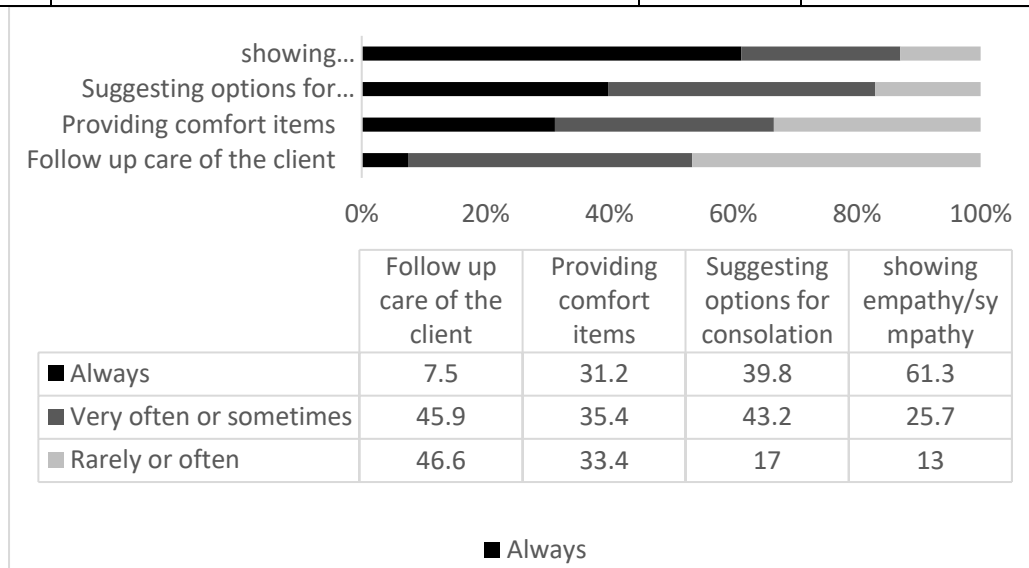


Figure 2. Providing emotional support to bereaved pet owners during companion animal euthanasia

Table 5. Results of ordinal logistic regression model examining the association between veterinarians' years in practice, and the extent to which veterinarians' use various client support practices

	Use of support practices to ease the stress of the client at an event of euthanasia	P value	Significance at 5%
01	Showing empathy and sympathy	0.003	Significant
02	Suggesting options for consolation	0.02	Significant
03	Providing comfort items	0.197	Not Significant
04	Follow up care of the client	0.307	Not Significant

Table 6. Distribution of responses to question items about moral criteria for choosing euthanasia

Question item	Should euthanize	Should not euthanize
1	75.5%	24.5%
2	19.1%	80.9%
3	17%	83%
4	20.7%	79.3%
5	5.3%	94.7%
6	2.1%	97.9%
7	3.2%	96.8%
8	3.2%	96.8%
9	3.2%	96.8%
10	2.1%	97.9%

Among the findings of general practices of veterinarians on euthanasia, the number of times which euthanasia was administered holds much importance. This study reveals that, approximately, the veterinarians administer euthanasia 0-2 times a month. According to a survey conducted in the US, the mean number of animals euthanized monthly by veterinarians in the southeastern United States was 7.53 [4], that is, 5 times as many as in Sri Lanka. These results depict that, Sri Lankan veterinarians and owners less frequently agree on deciding to euthanize companion animals than in the United States. Many Sri Lankan veterinarian seem to be find it difficult to make the decision to euthanize an animal alone. Therefore, as this study reveals that many veterinarians consult their colleagues before opting for euthanasia. As euthanasia is a stressful phenomenon, a quite ignorable percentage of veterinarians regret performing euthanasia

Findings of the present study widen the understanding of how Sri Lankan Veterinarians explore, understand and respond to clients' supportive needs during companion animal euthanasia, and how veterinarians' years in practice can impact the extent to which veterinarians presently use these practices.

Participants' years in practice (ie, participants' level of experience practicing veterinary medicine and companion animal euthanasia) was clearly shown to be positively associated with several of the above mentioned support and communication practices. This can be explained in such a way that, with more years in practice, veterinarians get more chances to perform euthanasia and thereby, they develop more confidence and competence in the process of administering companion animal euthanasia. Therefore, supporting the emotional needs of clients will be addressed more as the veterinarians proceeds in the clinical practice.

Analyzing situations which Sri Lankan veterinarians consider it appropriate to select euthanasia as a medical option for their patients are of greater importance. The results of data analyses revealed undeniable findings about their moral criteria in choosing euthanasia. First, two requirements seemed to be necessary for a majority of veterinarians to justify the choice of euthanasia for animals. One is "the animals are incurable and suffering," and the other is "the owners request to euthanize the animals." In the absence of either condition, the veterinarians were inclined to disapprove of choosing euthanasia. Among 10 question items about moral criteria for choosing euthanasia (Table 1), only question item A offers both conditions, and 75% of the veterinarians answered that they should euthanize the animals. On the other hand, for the other nine question items, which include one or none of the above

conditions, more than 80% showed clear disapproval of choosing euthanasia. However, a decrease in the animals' or the owners' QOL and the owners' inability to pay did not become foremost factors for them to justify the choice of euthanasia for the animals.

IV. CONCLUSIONS AND RECOMMENDATIONS

This study revealed significant information regarding the veterinarians' attitudes and perception toward euthanasia of companion animals in Sri Lanka. To the authors' knowledge, no previous studies have been carried out in Sri Lanka to investigate this area of interest in the veterinary medical field. The findings of this study can be utilized inevitably to grasp the situation surrounding euthanasia of companion animals in clinical settings in Sri Lanka. Many research questions still remain unanswered, such as about owners' views of euthanasia and veterinarians' stress management. In addition, this study did not evaluate how different religious/ethnic factors can affect, choosing the option of euthanasia. More culturally comparative and diversified studies are needed to examine and confirm the assumptions about how culturally different values influence differences in veterinarians' attitudes toward euthanasia between Sri Lanka and Western countries. In future studies, it is wished to elucidate these issues and seek a better way of managing companion animals' terminal care.

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CONE-BEAM COMPUTED TOMOGRAPHY AIDED PRE-ASSESSMENT OF MANDIBULAR AND MAXILLARY BONE QUALITY FOR DENTAL IMPLANTS -PHASE I

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Abstract

Dental implants play a major role in permanent tooth replacement. Cone Beam Computed Tomography (CBCT) can be used to acquire detailed volumetric image data of the maxillofacial region for diagnostic and pre-surgical planning of dental implants. The purpose of this study was to design and evaluate a decision-making algorithm to quantitatively assess the alveolar bone density of the maxilla and mandible as a pre-implant assessment. CBCT images with edentulous maxillary and mandibular bone areas of 50 patients who have undergone CBCT scans in the Dental Hospital, Peradeniya were selected. Those CBCT images were imported to the developed algorithm to calculate the Hounsfield values (HU) derived from Grey Values (GV) and categorized into types of alveolar bone density as stated in the Misch bone density classification. Moreover, the same set of images was independently analyzed by two experts, and a manual assessment was

performed on alveolar bone density according to Misch classification. The algorithm was more effective at locating the edentulous bone areas selected by the examiner and analyzing the bone density. Both experts were biased only on 40 cases with each other (80%) out of 50 cases. The correlation between CBCT GV and CT numbers should be calculated when converting GV into HU. Even so, in our study, it was assumed that there is a linear relationship regardless of the kV due to the shortfall of proper calibration in the CBCT machine. Besides the two-expert biased optical assessment and lack of proper calibration of CBCT machine, the algorithm was competent to achieve overall reliability of 77.5% following the comparison of manual and algorithm assessed results.

Keywords: Cone beam computed tomography, Dental implants, Alveolar bone density

I.INTRODUCTION

The World Health Organization discloses complete or partial loss of natural teeth as a public health problem [1]. Bridges and

dentures were the only treatment for years for the people who had missing teeth. But in modern times, dental implants play a major role in treating tooth loss [2,3].

Proper implant treatment planning takes priority for dental implant success. In planning, dental imaging plays a major role [1]. The objectives of this study were to develop an algorithm to aid in decision-making as a pre-implant assessment and evaluate the reliability of the developed algorithm.

Cone-beam computed tomography has become a leading modality in dentistry which is set to revolutionize diagnosis and management of endodontic problems.

CBCT scanners which are associated with extraoral imaging generate three-dimensional scans of the maxillofacial region by reducing the radiation absorbed by the patients. With a single 360° synchronized rotation of the scanner around the patient, a three-dimensional volume of data is acquired keeping a direct relationship between the sensor and the source. The X-ray beam is cone-shaped and a spherical volume of data is acquired [1].

The ability to acquire detailed volumetric image data of the maxillofacial region for diagnostic and presurgical planning purposes is the major advantage of using CBCT in dental implantation. Besides, due to its relatively lower dose of radiation and higher imaging resolution compared to conventional computed tomography, compact size, ease-of-use, it is widely used to provide detailed information for the diagnosis of oral complications. CBCT provides a noninvasive method to describe maxillofacial structures and assesses bone mineral density [1,3].

Bone quality assessment should be strongly recommended during the pre-surgical implant-planning phase since in practice, it is difficult to radiographically determine the quality of alveolar bone available for proper

implant support. It is manifested that higher implant failure is more likely in poor bone quality. Therefore, it is crucial to assess the bone density quantitatively by reducing human error [2,4].

Hounsfield units derived from grey values are the means of precise and quantitative evaluation of the dimension and density of the alveolar bone [1]. An automated algorithm facilitates the quantitative assessment of the bone quality of mandibular and maxillary regions and thereby, predicts the prognosis of the implants and provides better and safe treatment to the patients [2]. This study aimed to develop a non-invasive method to help in assessing the bone density before the dental implants using Hounsfield Units(HU) derived from grey values(GV) as a quantitative measurement.

III. MATERIALS AND METHODS

This study was approved by the Ethical Review Committee of the Faculty of Allied Health Sciences and Faculty of Dental Sciences, University of Peradeniya. The raw data of 50 patients who had been undergone CBCT scans in 2018 and 2019 were randomly selected from the CBCT archive of Dental Hospital, Peradeniya according to the inclusion and exclusion criteria.

All the selected patients were aged between 25-45 years old. The images with bone density- degrading pathological conditions and complete or partial dentures were excluded during the selection. All the selected images were composed of optimum image quality and void of artifacts. The patients had been exposed using 89 kV and 6 mA in CBCT and the exposed field of view (FOV) was 150×135 (voxel size = 0.2-0.3 mm). They were saved to a separate

folder in the CBCT archive and burned into compact discs and copied to a personal computer.

Copied Digital Imaging and Communications in Medicine (DICOM) files were viewed using ImageJ software to choose the exact region of interest (ROI), in axial sections of the edentulous area of the alveolar bone. Axial cuts were carefully chosen to start and end the extraction of alveolar bone volume and to position the seed point from the image series using the developed MATLAB code.

Imported DICOM images were first contrast adjusted and loaded to an image array. Next, to perform segmentation and isolation of the edentulous region, user input was needed. This was achieved by planting one or more seed points in the edentulous region.

A 40 x 40 pixels cropping was done to extract the approximate region of interest from the image and loaded it into a new array. This array of images was then fed into a third-party image segmentation algorithm. Segmented images were in the form of binary, and were loaded to a separate array.

To count the average gray values within the isolated region, the following method was used. (Figure 1)

A new image array was made, to which the same set of images as mentioned above was loaded but just without performing contrast adjustments. Considering the set of segmented binary images exported from the segmentation algorithm, for each image the corresponding original image was selected, and for each pixel containing '1' in the binary image corresponding GV from the original images was read. This was done to the whole segmented image sequence.

Total of pixels with '1' from the binary images and the summation of GVs from corresponding pixels were assigned to separate variables. The average Gray Value of the isolated edentulous region is obtained as follows.

Average Gray Value(GV) =

$$\frac{\sum \text{GVs from corresponding pixels were assigned to separate variables.}}{\sum \text{Number of pixels with '1' from the binary images}}$$

Based on the linear correlation of the GVs and HUs, the value range of GVs (9500) was mapped to that of HUs (4096). Rescaled GV range was used to derive average Hounsfield Units (HU) from the average Gray Values(GV) [4].

$$HU = \frac{4096}{9500} \times \text{Average Gray Value(GV)}$$

Obtained averaged HU was used to categorize the isolated region according to Misch's classification: bone density of D1, > 1250 HU; D2, 850-1250 HU; D3, 350-850 HU; D4, 150-350 HU and D5, < 150 HU. The results were recorded in Microsoft excel sheet.

The same set of image series were subsequently submitted to two experienced radiographers and a dentist for the manual assessment of alveolar bone density. These images were viewed in coronal plane using Slicer (4.10.2) software and the outputs were recorded into Microsoft excel sheet as categories in which they belonged to Misch classification.

The reliability of the algorithm is then assessed considering the experts categorization as the ground truth.

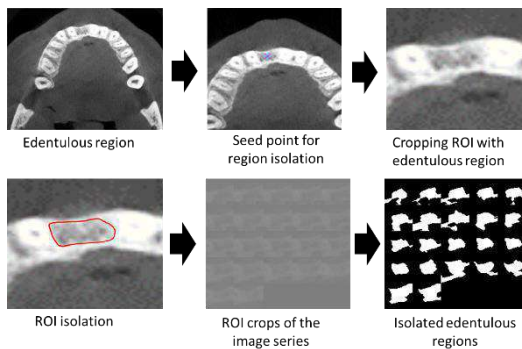


Figure 1. Image Processing Steps Using the Algorithm.

V. RESULTS AND DISCUSSION

Table 10. Typical data gathered by experts and the algorithm according to Misch classification and the percentiles

	Experts opinions match (AND)	Algorithm and both experts opinion match (AND)	Algorithm and any of the experts opinion match (OR)
Number of cases (n=50)	40	31	37
Percentile	40 / 50 * 100 = 80%	31 / 40 * 100 = 77.5%	37 / 50 * 100 = 74%

‘Algorithm AND’ indicates whether Expert1 and Expert2 both agree with the categorization of algorithm. (Figure 2)

if 1, Expert1 and Expert2 agrees with the categorization of the algorithm.

if 0, only one or none of the Experts agree with the categorization of the algorithm.

‘Algorithm OR’ indicates instances where any of the Experts agree with the categorization of the algorithm. (Figure 2)

if 1, Expert1 or Expert2 agrees with the categorization of the algorithm.

if 0, none of the Experts agree with the categorization of the algorithm.

The pre-evaluation of bone density is crucial, to place dental implantation. This study measured the bone densities at multiple edentulous sites using an automated algorithm.

When selecting images, it is important to strictly exclude any image with metallic artifacts. Even a small piece of metal in the scan volume may alter the bone density estimation considerably. When importing DICOM images into MATLAB working environment, it is wiser to import them in int16 or higher data type. Using a variable with lesser capacity may result in truncation of pixel values when calculating the average, causing inaccurate average GV, consequently inaccurate conversion to HUs.

Output of the algorithm is greatly varied by the modification of internal parameters of the algorithm. Categorization accuracy can be altered by modifying the parameters of segmentation algorithm, i.e. -attributes of Erode and Dilatation etc. though it has not used in this study.

It is shown in results analysis that out of 50 cases, both of the experts agree only on 40 cases with each other (80%). (Table 1)

Results obtained from the algorithm were compared to the expert’s opinion by three means.

- Both the experts and the algorithm agree upon the same bone category.

Sample size = number of cases considered (n=50)

$$\text{Algorithms score} = \frac{31}{50} \times 100\% = 62\%$$

- Both the experts and the algorithm agree upon the same bone category.

Sample size = Number of the cases where both experts agree (n=40)

('Algorithm AND' column in Table **Error! No text of specified style in document..11**)

$$\text{Algorithms score} = \frac{31}{40} \times 100\% = 77.5\%$$

$$\text{Algorithms score} = \frac{37}{50} \times 100\% = 74\%$$

Out of these comparisons it can be seen that the algorithm is able to achieve 77.5% accuracy when considering cases where both of the experts hold one opinion. Obviously, a larger sample size, a considerable number of experts who manually assess the bone density and CBCT machine calibration are more likely to increase the reliability of the results.

When the algorithm was given the freedom of variation of experts' opinion it achieved an accuracy of 77.5%. Additionally, the discrepancy between the opinions of the Expert1 and Expert2 caused a significant reduction of sample size.

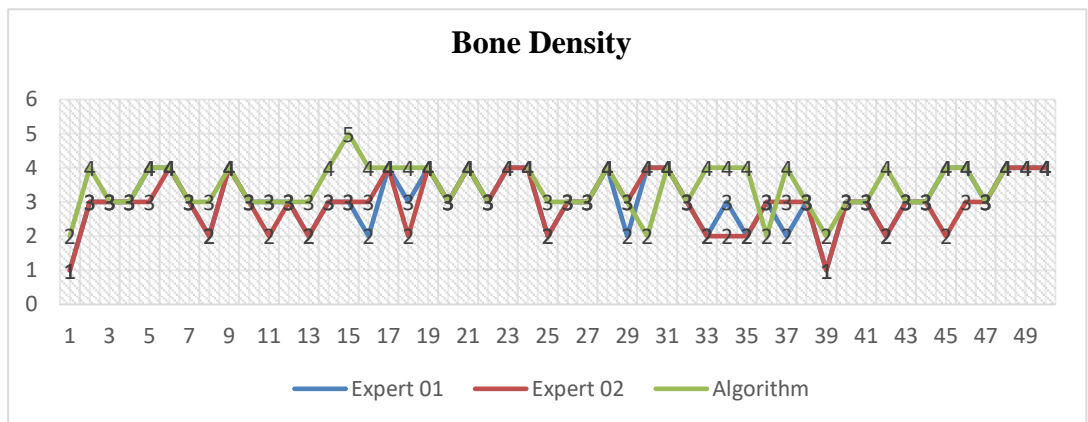


Figure 2. Variation of opinions on bone density classification according to the Misch classification from Expert 1, Expert 2 and algorithm

- Any of the two experts agree with the algorithm.

Sample size = number of cases considered (n=50)

('Algorithm OR' column in Table **Error! No text of specified style in document..12**)

VI. CONCLUSIONS AND RECOMMENDATIONS

This study shows the self-written algorithm scored 77.5% accuracy when compared to two independent experts in categorization of the alveolar bone according to the Misch classification. Further developments should

be carried out to increase the categorization accuracy.

A. Limitations

Although the produced algorithm is proved to have accuracy of 77.5% of its own, it was later found that our approach carried a significant flaw. Mah et al have carried a research on correlation of GV of CBCT machines to the HU of a medical CT machines. They have concluded that a linear relationship between GV and HU is found only in a narrow range of a CBCT machine. [2]

In our study it is assumed a linear relationship between GV and HU regardless of the kV used, which translated in to an unreliable categorization, regardless the backing of literature that said otherwise.

B. Recommendations

Repeating this research with a calibrated CBCT machine and a calibrated Medical CT machine will contribute to a classification with higher reliability.

In a future research, following will be a much better approach.

1. Obtain a phantom which contain at least 3 different standard densities.
2. Make exposures and take readings of standard densities from the CBCT machine for its entire kV range with fixed steps or 5 kVs.
3. Using the same phantom and for the same kV range, repeat the above step, using a medical CT machine instead of the CBCT machine.
4. For each kV step plot data.
 - 4.1 A = GV vs standard density.
 - 4.2 B = HU vs standard density.

5. Calculate offsets between A and B for each of kV step.
6. Plot offsets vs kV. A parabolic or inverse parabolic graph is expected.
7. Find the minima or the maxima of the plot.

GVs and HUs for the used machines behave linearly only in this kV setting. For all other kV settings suitable error corrections must be made to correctly translate GV in to HUs.

As well, incorporating a large sample size and more experts to assess the bone density are considered a better approach for a more reliable result.

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FOCUS AREA

**Information Communication Technology & Knowledge
Services**

A STUDY OF THE ROLE OF IT SOLUTIONS IN THE EXECUTION OF PUBLIC PROCUREMENT AND THE POSSIBLE ENVIRONMENT IMPACT

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Abstract

Procurement is the act of making the right product or service available to the right person at the right time at the right price in the right place. Procurement implication can impact on the overall cost of carrying out the decision in any organization. However, in this globalized world, almost all the processes are being becoming digitalized while manual systems are disappearing. In this context, procurement system has also been digitalized in most of the countries in the world. Therefore, e-procurement practices have been acquired such a popularity in the world. However, Sri Lanka is also moving gradually towards this digital processes and practices. In this context this study has been conducted to identify the level of e-procurement usage in public procurement in Sri Lanka. Accordingly, this study has been conducted within the universities in Sri Lanka. The core objective is to study the role of IT solutions in the execution of public procurement and the possible environment impact. This study is mainly based on the primary data which has been collected through three focus

group discussions which consist of four members in each group. The sample was made with the Procurement officers, administrative officers related to the procurement in the universities in Sri Lanka, Project Coordinators and Activity Coordinators of the projects which is going on within universities. All the gathered data has been analyzed and presented descriptively. As the findings of this study, it was revealed that the level of usage of e-procurement practices is not at a satisfactory level at universities in Sri Lanka and therefore the environmental impact also at a considerable level in Sri Lanka. Further, recommendations for increasing the level of e-procurement practices and the way of increasing the efficiency while eliminating delays using e-procurement practices has also been provided in each stage of procurement.

Keywords: Public procurement, e-procurement, Electronic procurement

I. INTRODUCTION

The term “procurement” has its roots in military logistics where formally acquired goods and services in 17th century. Simple meaning of the term “procurement” is Procurement is the process of finding and agreeing to terms, and acquiring goods,

services, or works from an external source, often via a tendering or competitive bidding process. The practice of procurement has become very crucial especially for government requirements as most of them are bulk purchase decisions. The organizations in current globe, has to be taken procurement related decisions as a

daily practice just because of the importance of this function [2].

By today, technology has been affected on almost everything in human lives. Technology has shaped our lives easier while introducing more simple and efficient ways of doing things. Accordingly, this situation has been influenced on the way of doing procurement practices as well. It has been identified the ways of conducting all the Manual procurement practices with the help of technology. It was revealed that doing thing electronically reduces the unnecessary delays. These kind procurement practices are well-known as “e-procurement” and the meaning of e-procurement is purchase and sales of goods, works and services through the internet as well as other information and networking systems. The e-procurement value chain consists of indent management, e-Informing, e-Tendering, e-Auctioning, vendor management, catalogue management, Purchase Order Integration, Order Status, Ship Notice, e-invoicing, e-payment, and contract management [4]. E-procurement in the public sector is emerging internationally. Most of the developed countries like India, Bangladesh, United Kindom, United States, Malaysia and Australia successfully using electronic procurement systems as a governmental practice [1]. This research was carried out in Sri Lankan universities. The main goal is to investigate the role of IT solutions in public procurement and their potential environmental impact.

Several literature evidenced that, Sri Lanka is still lacking behind in terms of the usage of electronic procurement in to practice. But, there is a few literature is available related to e-procurement usage Sri Lankan government sector organizations. This

paper has been developed as a case study with reference to the Universities in Sri Lanka to identify the level of e-procurement usage as a public sector organization [2].

The core objective is to study the role of IT solutions in the execution of public procurement and the possible environment impact. This study will distribute a significant knowledge regarding the usage of e-procurement within the universities in Sri Lanka. Therefore, the implications will be vital for other universities as well because most of the government university practices are homogeneous in nature. Other government universities in Sri Lanka are possible to just conduct a pilot study and identify the level of usage of e-procurement. Then they can practice the recommendations provided by this paper s it is [3].

It is also possible to generalize the findings once this study conducted with a sample which represent all the public universities in Sri Lanka. Further rooms are available to expand this study with the use of public sector business organizations as well.

II.MAERIALS AND METHODS

This study is mainly based on the primary data which has been collected through three focus group discussions which consist of four members in each group. The sample was made with the Procurement officers, administrative officers related to the procurement in the universities in Sri Lanka, Project Coordinators and Activity Coordinators of the projects which is going on within universities. All the gathered data has been analyzed and presented descriptively.

III.RESULTS AND DISCUSSION

There are two dimensions of procurement can be identified related to procurement in Universities in Sri Lanka. Some procurement activities are focused to purchase for the purpose of functioning of the university. This dimension will be common for any of the public sector organization in Sri Lanka. The purpose of other category is developing the university. There are various development projects financed by World Bank merely focus the development of the university both physically and outcome-based achievements. In this study, the term “procurement” refers both procurement related to general procurement of the university and university development projects.

All the development proposals should be submitted to the World Bank and after a rigorous evaluation the submitted university development proposals should be fulfill the World Bank criteria to be eligible to receive the allocated amount to execute the proposed development project in the university. Therefore, approvals and discussions should be made before the initiation of a development project. The approval receives from an external party to the university like World Bank, Asian Development Bank, voluntarily financing agency or the government.

Then the normal practice of all the universities before the Covid-19 pandemic was physically attending such events and maintaining minutes and other documents related to all the important discussions manually. In this phase telecommunication and letters were used to inform the event or meeting to participants. Most commonly used methods to communicate such meetings are email and registered post letters. However, as per the discussions it

was revealed that most of the universities are still following the traditional methods.

The bidding document is one of the core outputs of the Procurement Management Process. This document is used when requesting quotations from potential suppliers to purchase goods and services. This document consists of item list which are expected to procure, price schedule and a detailed description of the products, constructions or services which is expected to be purchase or construct using this bidding document. The preparation of bidding document is undertaking electronically.

The bid document clearance is the next step of university procurement. The ordinary practice of universities is, firstly various resource persons belong to the project team who have the knowledge regarding the items to be procure or works to be constructed, send the specifications to the relevant Project Assistant or the Project Coordinator via email. Then the Project Assistant accumulate all the specifications related to the certain procurement and prepare the bidding document. After that the hard copy of the bid document should be signed by the persons who have appointed to prepare the bidding document.

Specifications are exchange electronically via email all the time. But, the approval process takes considerable time because of the lectures of other appointed persons are not available at the university premises at the same day. Normally, there are three to five persons are appointing to prepare bidding documents and therefore, at least takes three to five days and it is certainly an unnecessary delay. However, it is possible and efficient to get all the confirmation through an email. The email thread can be used as the evidence. The Information and

Communication system has a huge responsibility here to have a mechanism to keep backups. However, most of the respondents stated that electronic signatures are not allowed in this phase. If it allows to use electronic signatures on bidding documents, after circulating the soft copy of the bidding document among the relevant personnel they can put their electronic signature after checking the accuracy of the details in the bidding document. This method will ease the task and it would be easy and effective with the view point of the Project Assistant or THE Procurement Assistant who is doing the coordination part. It is needed to maintain an electronic procurement file related to each procurement. Finally, this approved bidding document can be kept within that electronic file. These electronic practices will save not only the time and money but the environment also contributing to reducing the paper usage.

While the approval for bidding document is going on it is possible to appoint members for bid opening and Technical Evaluation Committee which is commonly known as TEC. Generally, these committees are appointing by the Vice Chancellor of the university and there is a manual procedure. But, it is possible and easy to appoint this committee electronically. Then, the appointed soft copy can directly be sent to the centralized office which is handling university procurement to further proceed. Or else, a clerical person has to go to the Vice Chancellors' office to submit procurement files for appointing committees and again go to Vice Chancellors' office to collect the appointed files. It takes several days to have the approval as well. Such unnecessary time and energy wastage can be minimized by using e-procurement.

Initially it was invited quotations only via registered post. But, when it has to re-invite quotations due to receive no quotations at first time or if the respective approving committee not give the approval for TEC report which has been awarded the items, quotations were recall via email. That practice indicates that there are no hard and fast rules that to invite quotations only by registered post. e-procurement is the procuring or buying of goods, services or construction work through electronic medium. e-bidding is the process of bidding through electronic medium. Although such electronic bidding procedures are available it was initiate to practice within Covid-19 pandemic. However, bidders have to send their quotations by registered post or courier service. It consists more than 300 papers for some quotations sent by suppliers. It is possible to imagine how much of a paper waste are the suppliers doing if they quote such quotations hundreds of times throughout the year. Allowing and accepting quotation via electronically would further save the environmental impact and money and energy of the suppliers as well.

In the normal practice, quotations are receiving time to time within the allocated period of time. Bid opening process is totally manual in Universities in Sri Lanka. But, bid opening process is totally run by electronic mode in the countries like India. For an instance, India has an electronic procurement hub called "Central Public Procurement Portal" and there is a comprehensive and very confidential method of bid opening. E-bidders can bid for procurement and bid opening occurs online. Summary of the bid opening also visible at the same time and it is very transparent method as well. E-procurement software is the enterprise system that automates and integrates the

spectrum of an organization's procurement cycle. A good solution for e-procurement software will increase the transparency of spending and approval processes.

Just like in other stages, approval for TEC report also has become manual at universities in Sri Lanka. Approval for TEC report is granted by Department Procurement Committee (DPC), Minor Procurement Committee (MPC) or Regional Procurement Committee (RPC). There is no any electronic procurement methods are following by this phase by Sri Lankan universities represented the selected sample. But, if it uses the electronic procurement methods it is possible to send the electronic version of the TEC report prepared by Technical Evaluation Committee to the relevant procurement committee (DPC, MPC or RPC) by as email within just a second. Then, the approval will also be received within a shorter period and the procurement process will be speedup. The clerical staff will not need to carry the files several times to get the approval.

Once received the approval for the report prepared by the Technical Evaluation Committee by considering the price proposals provided by the suppliers and other it writes purchase orders manually and send to the relevant suppliers by registered post. It takes several days to reach the purchase order to the supplier by post. However, if the purchase order sends to the relevant supplier via email the same expected result happens and it takes only two seconds to reach the purchasing order to the supplier. The current practice of the universities in Sri Lanka is before sending the purchasing order by registered post, scanned the purchasing order and forwarding to the relevant supplier by an email.

IV. CONCLUSIONS AND RECOMMENDATIONS

According to the findings of this study, it was revealed that universities in Sri Lanka is following traditional ways to conduct their procurement activities. Universities in Sri Lanka are relied on manual procurement processes and practices although some inefficiencies are there. Although some procurement officers are well aware of the inefficiencies, they have become muted just following the red tape bureaucracy embedded with the university system. Almost all the officers from top to bottom are concerning about their job security and any one will not go to break the existing rules and procedures. Anyone does not foreword to reveal the drawbacks or inefficiencies of the system. The benefits such as cost reduction, transparency, increasing productivity eradicating paperwork, upsurge transaction speed, consistent buying and reduced mistakes are possible to obtain with e-procurement. Therefore, the current paper is provided some insight to the decision-making authorities to take initiatives to move for e-procurement.

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FOCUS AREA

Water

REMOVAL OF LEAD IONS IN WASTEWATER USING THERMALLY REGENERATED DIATOMACEOUS EARTH FROM SPENT DIATOMACEOUS EARTH

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Abstract

In this abstract, thermally regenerated diatomaceous earth (DE) formed from spent diatomaceous earth (SDE) under 400 and 800 °C (SDE-400 °C and SDE-800 °C, respectively) was used as adsorbents to remove lead ions. SDE is mainly generated from food processing and brewery industries as an industrial waste of raw diatomaceous earth (RDE). This work also reports the effectiveness and efficiency of lead (Pb²⁺) ions removal from aqueous solution by the adsorbent of SDE. The surface morphology of SDE dry form was obtained by field emission scanning electron microscopy (FE-SEM) and indicates a well-arranged porous structure with some particles on the surface. Nitrogen adsorption-desorption analysis was performed at -196 °C for RDE and dry form of SDE and found that RDE has higher specific surface area and total pore volume of 3.70 m² g⁻¹ and 0.015 cm³ g⁻¹, respectively, than those of SDE dry form which has 2.22 m² g⁻¹ of specific surface area and 0.015 cm³

g⁻¹ of total pore volume. The favourable conditions for Pb²⁺ adsorption onto SDE were determined. The maximum lead adsorption was obtained, when the SDE dosage of 50 mg and contact time of 180 min at pH of 4.8, maintained for 50 mL of lead solution at 25 °C. The Langmuir and Freundlich adsorption isotherm models were used to interpret the equilibrium data of the investigated systems. The Langmuir model best described the adsorption characteristics of Pb²⁺ on RDE, SDE, SDE-400 °C and SDE-800 °C with maximum adsorption capacities of 92.60, 163.93, 208.33 and 322.58 mg g⁻¹. Moreover, the present study suggests the favourable application of SDE-800 °C as an effective material for the application in removal of Pb²⁺ from aqueous solutions for industrial wastewater treatment than other two adsorbents due to the presence of chemisorption and physisorption.

Keywords: Adsorption, Isotherm,

Lead (II), Spent diatomaceous earth,
Thermal regeneration

I. INTRODUCTION

Water is a fundamental requirement for life and main need for industrialization. Nowadays, water contamination is caused by the discharge of toxic effluents containing heavy metals from a wide range of industries. Lead is one of the industrial pollutants; possibly enter to the ecosystem through soil, air and water due to the industrial activities such as the manufacture of paint and pigments, Pb-containing pesticides, battery industries, petroleum refining, electrical and electronic products and lead smelters [1]. Therefore, these industrial effluents must be treated before discharge.

In this research, one of the cost-effective and environmental benignity methods of water purification, which is adsorption technology was applied using Diatomaceous Earth (DE) as the adsorbent because of its unique properties such as high silica content, high porosity, high surface area and chemical inertness [2]. Furthermore, the waste of DE i.e., Spent Diatomaceous Earth (SDE) was used to prepare thermally regenerated DE using thermal regeneration method under 400 and 800 °C (SDE-400 °C and SDE-800 °C, respectively). SDE is mainly generated from food processing and brewery industries as an industrial waste [3].

The main objectives of this study are to investigate the effectiveness and efficiency of ion removal by Raw Diatomaceous Earth (RDE), SDE, SDE-400 °C and SDE-800 °C, from aqueous solutions and further determine the efficiency of SDE by varying the parameters such as contact time, adsorbent dosage and the initial concentration of adsorbate.

II. MATERIALS AND METHODS

Chemicals

The Diatomaceous Earth (DE) and its waste, Spent DE (SDE) were supplied by Lion Brewery (Ceylon) PLC. Lead (II) Nitrate with min. purity 99%, was purchased from Sisco Research Laboratories Pvt. Ltd. (India). Sodium Hydroxide pellets (above 98.0%) were purchased from Daejung Chemicals & Metals Co., Ltd (Korea) and concentrated Nitric acid was obtained from Sigma Aldrich and both of them were used to adjust the pH of the solutions. All the chemicals used in the experiments were of analytical reagent grade and therefore used without further purification.

Instrumentation

The Field Emission Scanning Electron Microscopy (Hitachi SU6600 FE-SEM, Japan) and Nitrogen adsorption-desorption analyzer (ASAP 2010 volumetric analyzer, USA) were used for the characterization of SDE and both of RDE and SDE, respectively.

The thermal regeneration process of SDE under the temperatures of 400 °C and 800 °C was carried out in a laboratory furnace (Muffle Furnace-Human Lab DMF-12, Medi Lab & Research Technologies (Pvt) Ltd., Sri Lanka). The pH of each sample solution was adjusted using the Orion 420A pH meter (USA). The adsorbent and the adsorbate were contacted for a particular time using Lab Companion SK-600 Benchtop Shaker (Korea).

The concentrations of lead ions were recorded by Flame Atomic Absorption Spectrometer (Thermo M series AAS, Germany).

Methodology

1) Thermal regeneration of SDE: A certain amount of SDE sample (10.0000 g) was heated at a rate of 10 °C min⁻¹ to reach 400 °C and dwell at same temperature °C for 2h. Similar amount of sample was separately heated at a rate of 10 °C min⁻¹ to reach 800 °C and dwell at same temperature °C for 2h. Then the resulting products after thermal regeneration were cooled in a desiccator and labelled both prepared samples as SDE-400 °C and SDE-800 °C, respectively. Then four types of adsorbents (RDE, SDE, SDE-400 °C and SDE-800 °C) were used for the further experiments and for characterization measurements.

2) Adsorption isotherms: For the lead adsorption isotherm studies, batch experiments were conducted

using 50 mg of the adsorbent with initial concentrations ranging from 50 to 300 mg L⁻¹ under shaking for 180 min. So first, 50 mg L⁻¹ of lead nitrate solution was prepared into a 1000 mL volumetric flask, diluting an appropriate amount of the stock solution. Then (0.0500 ± 0.0001) g from each of the four adsorbents, i.e., RDE, SDE, SDE-400 °C and SDE-800 °C were measured and added to four bottles, separately, which contain (50.00 ± 0.05) mL of 50 mg L⁻¹ lead nitrate solution. The optimized parameters were maintained throughout this procedure. (pH of the solution = 4.8, volume of the solution = 50 mL, contact time = 3 h and stirring rate = 150 rpm). After settling the solutions were diluted as necessary (1:10 or 1:100 ratio) and carried out for the determination of remaining lead concentration using Flame Atomic absorption spectroscopy. The same procedure was performed by varying the initial concentrations with 100, 150, 200, 250 and 300 mg L⁻¹.

The Langmuir and Freundlich adsorption isotherm models were used to interpret the equilibrium data of the investigated systems **Table 13**.

The Langmuir model assumes that adsorption is monolayer and is dependent on the assumption that the adsorbent surface consists of active sites having a uniform energy [4].

Freundlich isotherm is one of the mainly used adsorption isotherms and it assumes that the adsorption of the adsorbate occurs on the heterogeneous surface by multilayer sorption and also assumes that the stronger binding sites are occupied first, and hence the binding strength decreases with the increasing degree of site occupation [7].

Table 13. Adsorption Isotherms

Langmuir Isotherm	
Linear	$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \quad (1)$
Non-Linear	$\frac{1}{q_e} = \frac{1}{K_L q_m} \times \frac{1}{C_e} + \frac{1}{q_m} \quad (2)$
Freundlich Isotherm	
Linear	$q_e = K_F C_e^{\frac{1}{n}} \quad (3)$
Non-Linear	$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (4)$

q_e – equilibrium adsorbed quantity (mg g^{-1})
 q_m – maximum adsorbed quantity (mg g^{-1})
 K_L – Langmuir constant (L mg^{-1})
 C_e – equilibrium concentration (mg L^{-1})
 K_F – Freundlich coefficient ($\text{mg g}^{-1} (\text{mg L}^{-1})^n$)
 $1/n$ – Intensity of the adsorption or surface heterogeneity ($0 < 1/n < 1$)

The amount of ion adsorbed at equilibrium (adsorption capacity), q_e (mg g^{-1}) by the adsorbent is

calculated from the following expression:

$$q_e = \frac{(C_0 - C_e)}{m} \times V \quad (5)$$

where C_0 is the concentrations of ion solution at initial in mg dm^{-3} , m is the mass of the adsorbent used in g , and V is the volume of the ion solution in dm^3 [6].

The essential feature of the Langmuir isotherm model can be expressed by means of a separation factor or equilibrium parameter (R_L), which is calculated according to the following equation;

$$R_L = \frac{1}{1 + K_L C_0} \quad (6)$$

The values of R_L indicate the type of biosorption isotherm, and there are four possibilities for the R_L value. (i) $0 < R_L < 1$ for favourable sorption, (ii) $R_L > 1$ for unfavourable sorption, (iii) $R_L = 1$ for linear sorption, and (iv) $R_L = 0$ for irreversible sorption [5].

The ion removal efficiency (percent ion removal) can be calculated by following expression [5]:

$$\% \text{ ion removal} = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (7)$$

III. RESULTS AND DISCUSSION

This research work is focused on the thermal regeneration of SDE due to the ease of performance, short time duration and mainly the utilization of SDE as an adsorbent after thermally regeneration is very scarce in literature. Furthermore,

Figure 2 illustrates a considerable colour difference among the four different adsorbents (RDE, SDE, SDE-400 °C and SDE-800 °C), used in this study. It was found in literature that sintering process could occur at 950 °C for SDE since SiO₂ and Al₂O₃ are the main components of it which have high melting point of 1710 °C and 2072 °C, respectively, and hence heating upto 400 °C and 800 °C is not affected to those main components.

A. Characterization of adsorbents

1) SEM Analysis: The surface morphology of SDE before lead adsorption, was analysed by Field emission scanning electron

microscopy (FE-SEM). Sample was mounted onto the sample stub using carbon tapes and the images were taken after gold sputter coating for 15 seconds.



Figure 2. The differences of the colour in four types of adsorbents: (a) RDE (b) SDE (c) SDE-400 °C and (d) SDE-800 °C

The images were obtained as shown in Figure 3 and that shows a well-arranged porous structure with some particles on the surface. Consequently, it is obvious that neither sintering temperature nor waste particles influence enough for closing pores, but the high temperature is capable of burning off or removing residues left in the internal pores. These openings are anticipated to enhance the adsorption of Pb²⁺ ions.

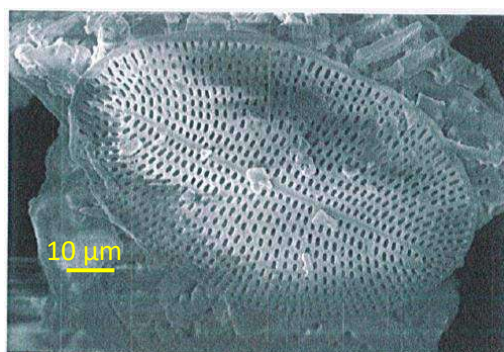


Figure 3. SEM image of SDE before adsorption

2) Nitrogen adsorption-desorption analysis: Nitrogen adsorption isotherms were measured at -196 °C on ASAP 2010 volumetric analyzer (Figure 4). Prior to the adsorption measurements, all samples were out gassed under vacuum at 110 °C for 2 hours.

The single-point pore volume (V_{sp}) was estimated from the amount adsorbed at a relative pressure (p/p^0) of ~ 0.98 . The pore size distributions (PSD) were calculated using adsorption branches of nitrogen adsorption-desorption isotherms by the improved KJS method calibrated for cylindrical pores. The Brunauer-Emmett-Teller specific surface areas (S_{BET}) were calculated from the N_2 adsorption isotherms in the relative pressure range of 0.05-0.2 using a cross sectional area of 0.162 nm² per nitrogen molecule (Table 14).

Table 14. Adsorption parameters for the DE and SDE samples studied

Sample	V_{sp} (cc/g)	V_{mi} (cc/g)	S_{BET} (m ² /g)	V_t (cc/g)
DE	0.014	<0.01	3.70	0.015
SDE	0.008	<0.01	2.22	0.010

V_{sp} - single-point pore volume calculated at the relative pressure of 0.98

V_{mi} – The cumulative pore volume of micropores, (pores below 2 nm) was calculated on the basis of the KJS method

S_{BET} – specific surface area calculated from adsorption data in relative pressure range 0.05-0.20

V_t - total pore volume calculated by integration of the PSD curve.

B. Effect of SDE dosage on Pb^{2+} ion removal

The amount of contact surface between an adsorbent and adsorbate solution plays a significant role in adsorption process. The influence of adsorbent dosage on lead ion removal was carried out by adjusting the SDE dosage from 10 to 100 mg while keeping the other parameters constant

(pH = 4.8, contact time = 180 min, and initial concentration of lead ion solution = 100 mg L⁻¹). From the results, the adsorption capacity of SDE was improved from 1.412 to 4.4860 mg g⁻¹ when the dosage was increased from 10 to 100 mg (Figure 5). These results could be mainly due to the fact that more active adsorption sites become available with the increasing adsorbent dose, allowing more adsorbate ions to adhere to it [8]. However, there was no observable change in the adsorption capacity after 50 mg of adsorbent dosage, suggesting that it had reached its saturation level as there

were no lead ions available to be adsorbed at higher dosages, due to the assumption based on that the number of adsorption per unit mass of adsorbents remains constant [5]. Thus, the 50 mg of adsorbent dosage was optimized and selected for further experiments. However, it is obvious that the adsorption capacity or the metal ion removal efficiency increases with the increase in adsorbent dosage due to the availability of active adsorbent sites [9]. Therefore, this study indicated that 50 mg of the adsorbent is sufficient to adsorb the maximum ions with the maximum adsorption capacity of 4.415 mg g⁻¹ at an optimum pH of 4.8.

C. Effect of contact time on Pb²⁺ ion removal

The effect of contact time on the adsorption capacity of SDE was studied by varying it from 30 to 240 minutes, keeping other parameters constant at their optimum values. Figure 6 shows the effect of contact time on the adsorption capacity of the adsorbent. As contact time increases adsorption capacity also initially increases due to the availability of freely accessible active sites with strong attractive forces between the lead molecules and the adsorbent and then gradually attains almost an equilibrium in nearly 180 minutes (3 hours) and remains more or less constant thereafter as there were no considerable amount of lead ions present to adsorb on to SDE [10].

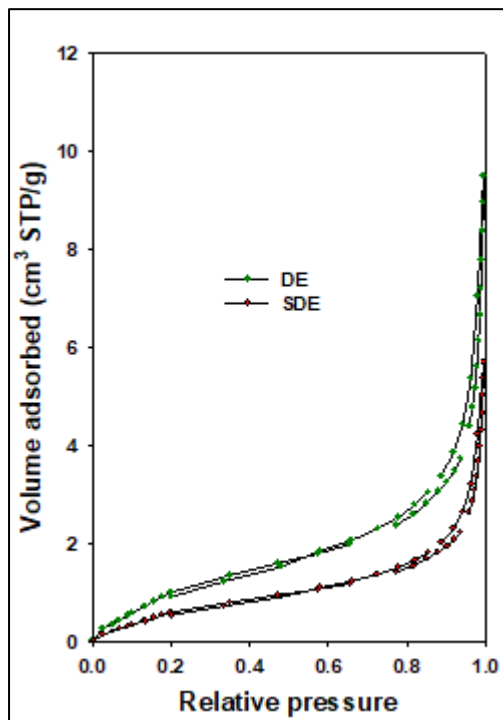


Figure 4. Nitrogen adsorption-desorption isotherms of DE and SDE

D. Adsorption isotherms

Adsorption is usually described by isotherm which is a graph or a relation between the amounts of adsorbate adsorbed on the surface of adsorbent and pressure at a constant temperature [11].

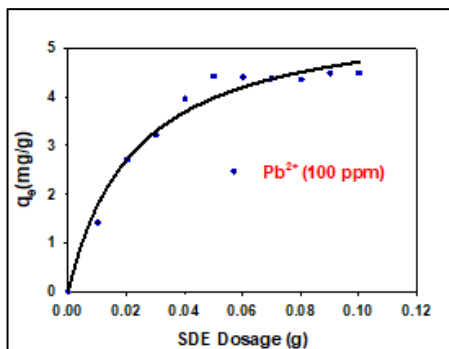


Figure 5. Effect of adsorbent dose on the adsorption capacity of SDE (initial lead ion concentration = 100 mg L⁻¹, pH = 4.8, contact time = 180 min, and stirring rate = 150 rpm)

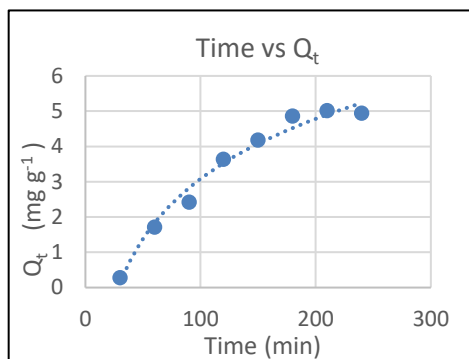


Figure 6. Effect of adsorbent dose on the adsorption capacity of SDE (initial lead ion concentration = 100 mg L⁻¹, pH = 4.8, contact time = 180 min, and stirring rate = 150 rpm)

Moreover, there are several isotherm models that were studied by different scientists and this study is mainly focused on two different adsorption isotherms.

In this study, two main adsorption isotherm models of Langmuir and Freundlich were applied to the experimental data.

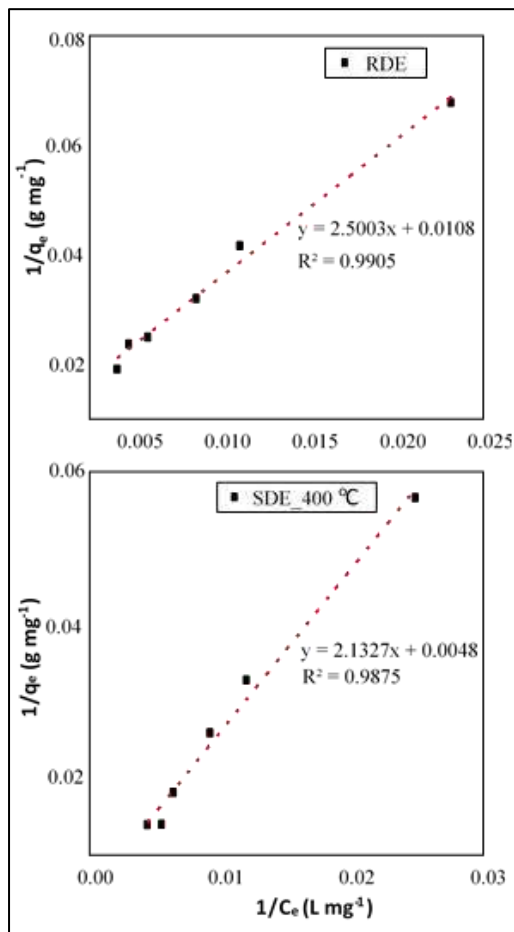


Figure 7. Langmuir isotherm of Pb²⁺ ions adsorption on RDE and SDE-400 °C

Equation (2) was used to obtain Langmuir isotherm for the adsorption of Pb²⁺ using the adsorbents of RDE, SDE, SDE-400 °C and SDE-800 °C. Figure 7 and Figure 8 show the Langmuir isotherm of experimental data for Pb²⁺ on four different adsorbents. Equation (6) was used to calculate the value of dimensionless equilibrium parameter R_L , which is referred to as separation factor or equilibrium parameter [10]. In this study, Langmuir isotherm indicated

favourable conditions for the adsorption of Pb²⁺ ion on the adsorbents, because R_L was between zero and one [10].

Equation (4) was used in order to apply the experimental data to Freundlich isotherm and Figure 8 and Figure 9 show Freundlich isotherm of the experimental data on Pb²⁺ ion removal. The $1/n$ values of four different adsorbents falls between 0 and 1 (Table 15), which indicate the favourable adsorption of lead ions on those adsorbents [5].

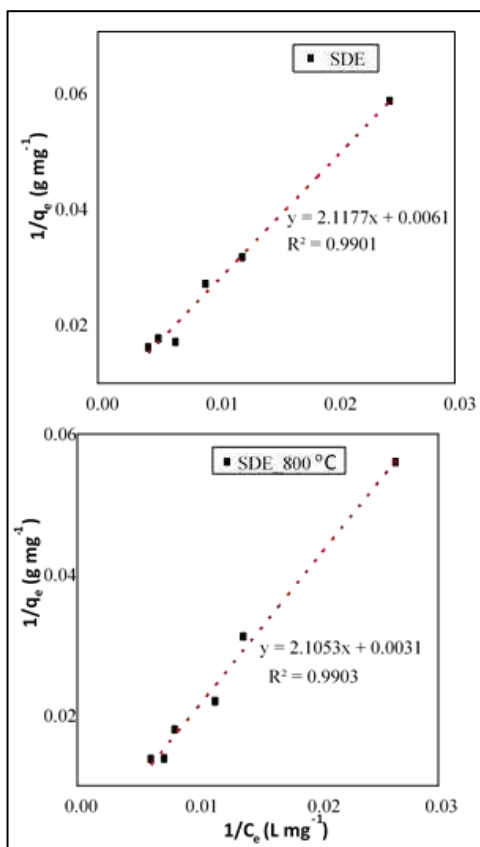


Figure 8. Langmuir isotherm of Pb²⁺ ions adsorption on SDE and SDE-800 °C

Freundlich isotherm assumes not only that the adsorption of the

adsorbate occurs on the heterogeneous surface by multilayer sorption but also that the stronger binding sites are occupied first, and that the binding strength decreases with the increasing degree of site occupation. The K_F (mg g⁻¹)(mg L⁻¹)ⁿ or (mg g⁻¹(L mg⁻¹)^{1/n}) present in the equation (4) is referred as Freundlich coefficient which is a constant indicative of adsorption capacity of the adsorbent, and the dimensionless constant $1/n$ indicates the intensity of the adsorption or surface heterogeneity and its value ranges between zero and one.

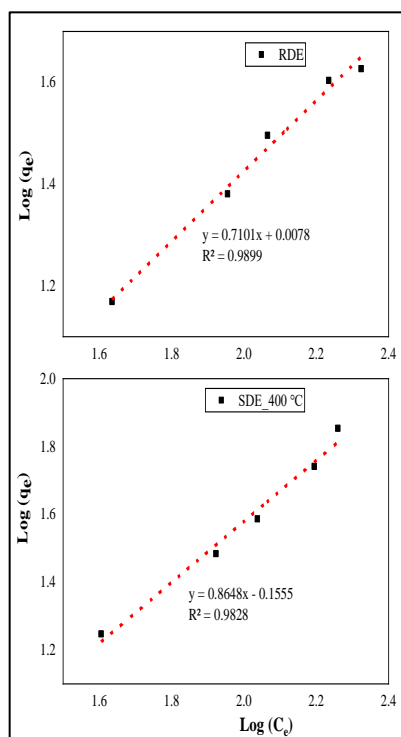


Figure 9. Freundlich isotherm of Pb²⁺ ions adsorption on RDE and SDE-400 °C

When the K_F value increases, the adsorption capacity of adsorbent for a given adsorbate increases. The plot of $\log w_e$ versus $\log C_e$ gives a straight line with a slope of $1/n$ and the value of $\log K_F$ can be obtained by the intercept, indicating multilayer sorption capacity.

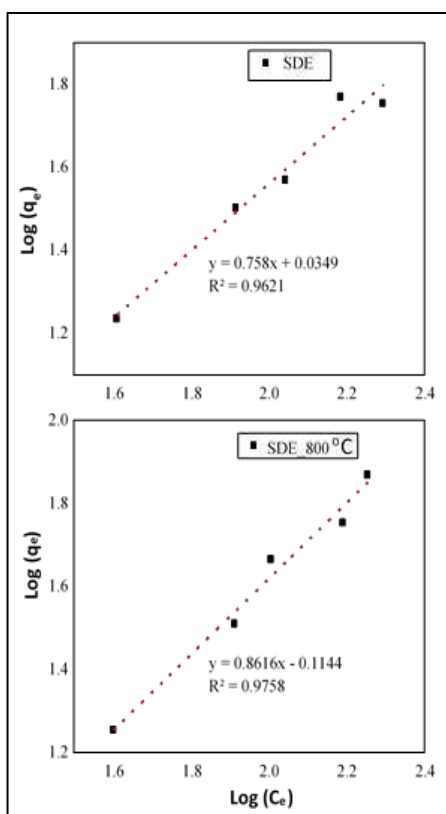


Figure 10. Freundlich isotherm of Pb^{2+} ions adsorption on SDE and SDE-800 °C

The correlation coefficients of Freundlich isotherm are, however, less than those of Langmuir isotherm as can be seen from Table 15.

The plots of $1/q_e$ versus $1/C_e$ for the sorption of Pb^{2+} ions on four types of adsorbents gave straight lines with high correlation coefficients which

indicate good fit of data to the Langmuir isotherm than the Freundlich isotherm (Figure 7 and Figure 8). The better fitness of the data to Langmuir adsorption isotherm suggests that the adsorption is monolayer and is dependent on the assumption that the adsorbent surface consists of active sites having a uniform energy [5]. adsorbent surface consists of active sites having a uniform energy[5]. Table 15 presents the calculated parameters for Langmuir and Freundlich isotherms and implied that Freundlich isotherm models could not be used to explain the adsorption process of Pb^{2+} ions on four types of adsorbents (RDE, SDE, SDE-400 °C and SDE-800 °C).

Table 15. Isotherm model parameters for the adsorption of Pb^{2+} onto RDE, SDE, SDE-400 °C and SDE-800 °C

Isotherm		RDE	SDE	SDE-400 °C	SDE-800 °C
Langmuir	q_m ($mg\ g^{-1}$)	92.59	163.93	208.33	322.58
	K_L ($L\ mg^{-1}$)	0.0043	0.0029	0.0023	0.0015
	R^2	0.99	0.99	0.99	0.99
Freundlich	$1/n$	0.7101	0.758	0.8648	0.8616
	K_F ($mg\ g^{-1}$) ($L\ mg^{-1}$) ^{$1/n$}	1.0181	1.0839	0.6990	0.7684
	R^2	0.99	0.96	0.98	0.98

IV. CONCLUSIONS AND RECOMMENDATIONS

This research work is focused on the synthesis of silica adsorbent using thermal regeneration of Spent diatomaceous earth (SDE) under 400 °C and 800 °C due to the ease of performance, short time duration and mainly the utilization of SDE as an adsorbent after thermally regeneration is very scarce in literature.

The surface morphology of SDE before lead adsorption, was analysed by Field emission scanning electron microscopy (FE-SEM) and that indicates a well-arranged porous structure with some particles on the surface. Nitrogen adsorption-desorption analysis was performed at -196 °C for Raw DE (RDE) and dry form of SDE and found that RDE has higher specific surface area and total pore volume of 3.70 m² g⁻¹ and 0.015 cm³ g⁻¹, respectively, than those of SDE dry form which has 2.22 m² g⁻¹ of specific surface area and 0.015 cm³ g⁻¹ of total pore volume.

A series of adsorption experiments were conducted as a function of adsorbent dosage and contact time and it was found that the adsorption capacity for Pb²⁺ ions increased with the increasing adsorbent dosage and the contact time and then gradually attains almost an equilibrium at a respective particular point onwards. These results could be mainly due to the fact that more active adsorption sites become available at initial and with the increasing adsorbent dose,

allowing more adsorbate ions to adhere to it and afterwards it may be reached to its saturation level as there were no adsorbates available to be adsorbed at higher dosages and time passes. Therefore, the favourable conditions for Pb²⁺ adsorption onto SDE were determined to be the adsorbent dosage of 50 mg and contact time of 180 min at pH of 4.8, respectively.

The adsorption data of Pb²⁺ on the adsorbents of RDE, SDE, SDE-400 °C and

SDE-800 °C were analysed using the Langmuir and Freundlich adsorption isotherm models and those adsorption data fitted well with the Langmuir model with a good correlation coefficient value which indicates that the adsorption is monolayer and is dependent on the assumption that the adsorbent surface consists of active sites having a uniform energy. Langmuir isotherm indicated the favourable conditions for the adsorption of Pb²⁺ on those adsorbents, since R_L value was between zero and one. Moreover, observed experimental data of Freundlich isotherm on Pb²⁺ ions also indicated a favourable adsorption of lead ions on those adsorbents because of the 1/n values of four different adsorbents falls between 0 and 1.

The Langmuir model is owing to the maximum adsorption capacities for Pb²⁺ on RDE, SDE, SDE-400 °C and SDE-800 °C as 92.60, 163.93, 208.33 and 322.58 mg g⁻¹, respectively.

The presented study suggests the favourable application of SDE-800 °C as an effective material for application in the removal of Pb²⁺ from aqueous solutions for industrial wastewater treatment than other three adsorbents due to the presence of both chemisorption and physisorption. Chemisorption probably occurs due to the breakdown of C-O-C bonds to form -C-OH bond when it was heated to high temperature and hence the interaction between adsorbate and the adsorbent surface occurred probably via electrostatic attractions and H bonds. On the other hand, physisorption may occur due to Van der Waals forces between the adsorbate and the pores available on the adsorbent surface.

Abbreviations and Acronyms:

AAS - Atomic Absorption Spectrometer
DE - Diatomaceous Earth
FESEM - Field Emission Scanning Electron Microscopy
RDE - Raw Diatomaceous Earth
SDE - Spent Diatomaceous Earth

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