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# 9TH YSF SYMPOSIUM

13th November 2020

Organized by

Young Scientists Forum

National Science and Technology Commission

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## Message from the Chairperson,
## National Science and Technology Commission

I am extremely pleased to note that the Young Scientists Forum (YSF) of the National Science & Technology Commission (NASTEC) has organized the Annual Research Symposium for the 9th consecutive year.

The concept of the Young Scientists Forum (YSF) has had emanated from the World Conference on Science for the 21st Century, held in Budapest, Hungary in 1999, under the aegis of the United Nations Educational, Scientific & Cultural Organization (UNESCO) and the International Council for Science (ICSU), France. YSF of NASTEC was established in the year 2000 having a similar mandate where young scientists (45 years or below) get together discuss many current issues pertaining to the country & world and also emerging technology. The YSF Symposium which was initiated in year 2012 has become a popular event as a forum that gives an opportunity for young scientists to present their research work and discuss with the scientific community.

I am very happy to note that about 60 extended abstracts under the focus areas of National Research & Development Framework (NRDF) are to be presented during the symposium in parallel sessions. These research papers address important areas relevant to socio- economic development including sectors of Food, Nutrition & Agriculture, Health & Indigenous Knowledge, Energy & Environment, Textile & Apparel, and Information and Communication Technology (ICT).

As per the Government vision towards a digital society, the presentations of the young scientists would lead to new platforms to interact with society through IOT. This would also create platform to develop new research collaborations.
I would also take this opportunity to encourage the promising young researchers gathered here not only focus on research but also in innovations & commercialization towards entrepreneurship and utilize their academic expertise to develop small and medium industry. This would create job providers rather than job seekers in the country.

I wish to thank the NASTEC officials & YSF Steering Committee for organizing this symposium successfully and wish you all the best in your future endeavors.

**Professor Kshanika Hirimburegama**

**Chairperson**
**National Science and Technology Commission**

## Message from the Acting Director
## National Science and Technology Commission

It is with great pleasure that I convey this message as the Acting Director/ CEO of the National Science and Technology Commission (NASTEC) at the inauguration of the 9th YSF Annual Research Symposium organized by the Young Scientists Forum (YSF) of NASTEC.

YSF Symposium which has been initiated in 2012 was held successfully since its inception. This year the symposium is focused on promoting Research & Development (R & D) with relevant interventions identified under the ten thrust areas of the National Research & Development Framework (NRDF), which was developed by the NASTEC under the auspicious of the then Ministry of Technology & Innovation and in consultation with a large number of relevant experts in the scientific community. The NRDF is a cabinet approved national R & D framework that encourages young scientists and promising researchers of the country to carry out research on areas identified in NRDF that support national priorities of the country. I am pleased to note the number of research publications related to thrust areas of Health, Food, Nutrition & Agriculture, Environment, Energy, Basic Sciences, and Emerging Technologies & Indigenous Knowledge being presented in this symposium which has clear link with national priorities.

It is my fervent hope that the young scientists gathered at this Symposium will not only share their knowledge and experiences of novel research interventions, but also will mobilize their collective intellect to improve the performance of Science, Technology and Innovation (STI) sector enabling the creation of a knowledge based economy.

While wishing good luck with the proceeding of the sessions, I kindly request the young scientists and researchers to discuss the national R & D priorities in line with the Saubhgyaye Dekma; the National Policy Framework during the deliberations enabling to meet the national targets of the STI sector.

**Mrs. Nazeema Ahamed**
**Acting Director**
**National Science and Technology Commission**

## Message from the Steering Committee Chairperson
## Young Scientist Forum

It gives me great pleasure to issue this message on the occasion of the 9th Annual Symposium of the Young Scientist Forum as the chairperson of the Steering Committee. Given that we are living in a more dynamic world which is changing at a great speed, we have to rely more on the energy, perspectives and new knowledge of the youth. It is increasingly become necessary that young generation need to be equipped with requited skills that facilitate them to adapt and prosper in a changing world. It is becoming more and more necessary to create novel systems that should be in support of young generation and to empower them to shape the future they want without letting young generation react to the future that others create for them. In this way confronting the challenges of the modern world of science would be easier.

The objective of holding this symposium is to provide an opportunity to young scientists to discuss scientific challenges and share their research findings on key areas of Food, Nutrition and Agriculture, Environment, Energy, Textiles and Apparel, ICT, Basic Sciences and Emerging Technology etc. The symposium is in line with the mandate of NASTEC for engagement of youth in positive activities and also serve as a platform for young scientists to promote networking and offer unique opportunity for collaborative research by sharing their recent research findings, future plans and their research interests. This kind of opportunities ensures that young scientists will be exposed early on to the best standards of research and learn a rigor that will positively influence their future discoveries.

At this occasion, I take the pleasure to convey my sincere gratitude to Prof. K. Hirimburegama, the Chairperson of NASTEC and Mrs. Nazeema Ahamed, the Acting Director of the NASTEC for continues support extended for all the YSF activities including the 9th YSF Symposium. I am indebted to the contribution provided by the panel of reviewers in reviewing and maintaining the standards of the EA's presented in the annual symposium. I wish to thank to the steering committee members of YSF for their dedicated and tireless efforts made for this accomplishment. I wish to extend my thanks to the Editorial Board and the Chief Editor for their invaluable effort made for producing the Proceedings of the symposium. 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 9th Annual symposium of the YSF a grand success.

**Dr. Lasantha K. Weerasinghe**
**Chairperson - YSF Steering Committee**

## Foreword from the Editors

It is with great pleasure, the Young Scientist Forum (YSF) present the proceedings of the 9th 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 81 extended abstracts received for the year 2019, 51 submissions were selected through a double-blind review process. The materials submitted by the authors were reviewed by two expert reviewers in the relevant field and have been edited by editorial board of the YSF. The views expressed in extended abstracts 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 Prof. K. Hirimburegama, the Chairperson of NASTEC and Mrs. Nazeema Ahamed, the Acting Director of the NASTEC for funding and facilitating the events of YSF with great enthusiasm. NASTEC coordinator Ms. Thilini Munagamage and NASTEC staff, and the members of the YSF Steering Committee are also acknowledged for the immense support rendered in organizing the symposium and compilation of the proceedings.

We wish the 9th YSF symposium a great success and extend warm wishes to all the authors.

**The Editorial Board**
**9th YSF Symposium Proceedings**

# EXTENDED ABSTRACTS

## FOCUS AREA

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

# ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *Litsea iteodaphne* ("KALU NIKA") AGAINST CLINICAL ISOLATES OF METHICILLIN RESISTANT *Staphylococcus aureus*

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## Introduction

The use of higher plants to treat infectious diseases is an age-old practice in a large part of the world population, where there is reliance on traditional medicine for a variety of diseases [1]. Interest in plants with antimicrobial properties has invigorated as a consequence of existing problems associated with the use of antibiotics [2]. There has been an increased apprehension in studying antimicrobial properties of plants after emergence of antibiotic resistance to current antibiotics as a result of antibiotics misuse. Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) isolates have increased greatly during the last decades in the community [3].Medicinal plants have been used as remedies for communicable diseases in many tropical countries, providing a rationale for discovering natural products for the treatment of MRSA infection. *In vitro* antimicrobial screening permits the selection of plant extracts with potentially useful properties to be used for further chemical and pharmacological studies. *Litsea iteodaphne* which is known in Sinhala as "KaluNika" is a rare endemic plant grown in Sri Lanka. *L. iteodaphne* has been used in traditional ayurveda medicine in the treatment of arthritis, boils, cough, ulcers and infections in the ear. Traditional use of this plant suggests its possible antimicrobial properties, but its antimicrobial activity against MRSA has never been investigated before. Therefore, the present study was designed to investigate the *in vitro* antibacterial potential of ethanol, hexane and aqueous extracts of *L. iteodaphne* against MRSA pathogenic bacteria and to screen the phytochemicals responsible for this antimicrobial activity providing an option to address the urgent issue of bacterial resistance to antibiotics.

## Materials and Methods

*Sample collection*

*Litse aiteodaphne* plant leaves were collected from Southern region in Sri Lanka. Plants were authenticated at the National Herbarium, Botanical Gardens, Peradeniya, Sri Lanka. The leaves were washed in water, air-dried in the oven and stored in a sterile air-tight container until further use.

*Extract preparation*

Ethanol and hexane extracts were prepared using coarsely powdered leaves soaked in ethanol and *n*-hexane solvents respectively. Aqueous extract was prepared by refluxing coarsely powdered leaves. The solvents were concentrated and the crude samples were dissolved in 10% DMSO (Dimethyl Sulphoxide) to prepare 400, 40 and 4mg/ml concentrations of ethanol, hexane and aqueous extracts and stored in the refrigerator.

*Tested organisms*

The extracts were tested against20 methicillin resistant *Staphylococcus aureus* (MRSA 1-20) clinical isolates obtained from the Department of Microbiology, Faculty of Medicine, University of Ruhuna.

*Disc diffusion assay*

Initial antibacterial activity was screened using the disc diffusion method. The bacterial colonies were inoculated in normal saline and cell suspensions were adjusted to 0.5 McFarland turbidity standards. Sterilized Whatman No.1 filter paper discs were impregnated with10 µl of different concentrations of plant extracts and solvent blank (10 % DMSO) and placed on the inoculated plates with vancomycin as the positive control. The plates were incubated and the diameters of the zones of inhibition were measured to screen the antibacterial activities.

*Determination of MIC*

The MICs of the plant extracts were determined by broth dilution assay using sterile96-well microtitre plates adhering to Clinical and Laboratory Standards Institute, M07-A8 [4]. The plant extracts were serially diluted to produce a concentration series from400 mg/ml to 0.0256 mg/ml. Vancomycin IV was used as the positive control and 10% DMSO was the negative control. Inoculum was added into each well containing 90 µl plant extract in the dilution series, and mixed. The plates were incubated in an ambient air incubator. Absorbance of the plates was measured at 630 nm and MIC was determined.

*Phytochemical Analysis*

Qualitative phytochemical tests to detect the presence of tannins, cardiac glycosides, reducing sugars, alkaloids, phenolic compounds, cyanogenic glycosides, saponins, and flavonoids were carried out using standard procedures [5]

*Statistical analysis*

Mean and standard error of mean (SEM) were calculated. One-way analysis of variance (ANOVA) with post-hoc analysis (Bonferoni) was used to compare the means.

**Results and Discussion**

The most significant inhibition zones for the MRSA strains were given by ethanol extract followed by hexane and water extracts. Zones of inhibition ranging from 6.1mm to 10.9 mm were obtained for 400mg/ml of ethanol extract while zones of inhibition ranging from 6.7 mm to 10.8 mm were obtained for the same concentration of the hexane extract. For the 400 mg/ml concentration of the aqueous extract, zones of inhibitions were ranged from 6.4 mm to 8.6 mm. Out of the three extracts tested, 400 mg/ml concentration of both ethanol and hexane extracts were sensitive against all the MRSA strains. Significant zones of inhibition of 10.9 mm (MRSA 14), 10.5 mm (MRSA 4), 10.1 mm (MRSA 8) and 9.7 mm (MRSA 7) were obtained for 400mg/ml of ethanol extract while zones of inhibition of 10.8 mm (MRSA 5), 10.1 mm (MRSA 13),9. 8 mm (MRSA 2) and 9.1mm (MRSA 19) were obtained for the same concentration of the hexane extract. For the 400 mg/ml concentration of the aqueous extract, zones of inhibitions of 8.6 mm (MRSA 2), 8.1 mm (MRSA 13), 7.9mm (MRSA 18) and 7.8 mm (MRSA 19) were obtained (Figure 1).

MIC values of the ethanol extract ranged from 0.64 mg/ml to 0.0256 mg/ml. MIC values of both hexane and water extract ranged from 3.2 mg/ml to 0.0256 mg/ml. Among the three extracts majority of the organism were inhibited by the 0.0256 mg/ml concentration of the ethanol extract. Therefore, from the three extracts tested against above twenty MRSA organisms ethanol extract of *L. iteodaphne* showed the highest antimicrobial activity.

Mean (SEM) of MIC obtained for ethanol, hexane and water extracts were 0.12 (0.05), 1.39 (0.34) and 0.63 (0.32) mg/ml respectively. Mean MIC of ethanol extract is significantly ($p$=0.002) lower than hexane extract. There was no statistically significant difference between mean MIC of aqueous and ethanol extract ($p$ =0.600). Further, mean MIC difference was not significant between aqueous and hexane extract ($p$=0.187).

Out of the main phytochemicals tested only tannins, cardiac glycosides, reducing sugars, phenolic compounds, saponins and flavonoids were found to be present in *L. iteodaphne* leaf extracts. Compounds like tannins found in plant cells are potent inhibitors of hydrolytic enzymes used by pathogenic bacteria. Phenolic compounds present in the extract of this plant are powerful inhibitors of microbial growth. Glycosides eliminate the poisonous compounds from the body. Thus the presence of such phytochemicals may be correlated with the fact that crude extracts of the plant showed antibacterial activity against the MRSA strains used in the study.

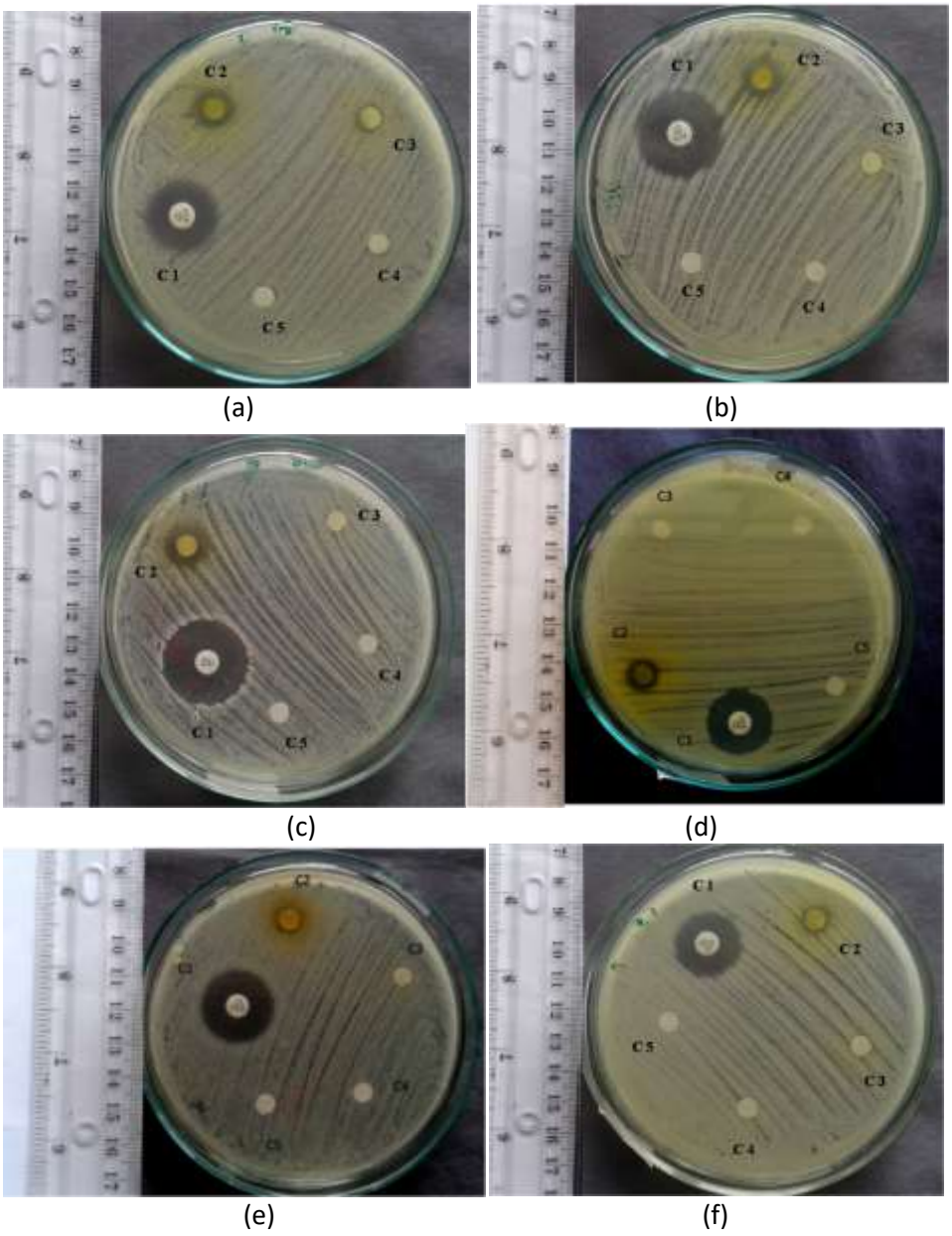


**Figure 1**. Some positive results of the disc diffusion assay. (a) Ethanol extract of *L. iteodaphne* against MRSA 14 bacteria(b) Ethanol extract of *L. iteodaphne* against MRSA 4 bacteria (c) Ethanol extract of *L. iteodaphne* against MRSA 8 bacteria(d) Ethanol extract of *L. iteodaphne* against MRSA 7 bacteria (e) Hexane extract of *L. iteodaphne* against MRSA 5 bacteria (f) Aqueous extract of *L. iteodaphne* against MRSA 2 bacteriaC1: positive control; C2: crude extract; C2: 10-fold dilution of crude extract; C3: 100-fold dilution of crude extract; C5: negative control.

**Conclusions and Recommendations**

From the ethanol, hexane and aqueous extracts tested against MRSA isolates, ethanol extract of *L. iteodaphne* showed the highest antimicrobial activity with a MIC of 0.0256 mg/ml. Presence of phytochemicals such as tannins, cardiac glycosides, reducing sugars, phenolic compounds, saponins and flavonoids in the plant extract suggests that these phytochemicals may be responsible for the antibacterial activity. Since this activity was given by the crude extract of the plant, fractionation and isolation of active compounds from *L. iteodaphne* may increase its therapeutic potential in the treatment of infections caused by methicillin-resistant *S. aureus*.

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# ACUTE AND SUB-ACUTE TOXICITY STUDY OF *Acronychia pedunculata* LEAVES

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## Introduction

*Acronychia pedunculata* ("Ankenda" in Sinhala, Family: Rutaceae) is a small evergreen tree found in Sri Lanka and the leaves, stems, roots and fruits have been used for centuries in folk medicine for the treatment of various diseases. Our previous studies have shown that 70 % ethanol extract of leaves of this plant has significant ($p < 0.05$) acute and chronic anti-inflammatory activity on carrageenan-induced rat hind paw oedema modal and adjuvant induced arthritis rat model respectively. Further it was found that it has significant ($p < 0.05$) anti-histamine, anti-nociceptive, *in-vivo* and *in-vitro* anti-oxidant, nitric oxide scavenging activities as well as prostaglandin $E_2$ inhibitory activity. Although, it shows different biological actions no scientific data are available regarding its potential adverse effects. The lack of information regarding the toxicity of this plant limits the possible long-term use in chronic disease conditions. Hence, in the present study an attempt has been made to evaluate the acute and sub-acute toxicity of *A. Pedunculata* leaves.

## Material and Methods

*Plant materials*

Fresh *A. pedunculata* leaves were collected from Kottawa area in the district of Colombo. It was authenticated and a voucher specimen (KMR002) was deposited at National Herbarium, Department of National Botanic Garden, Peradeniya, Sri Lanka.

*Ethical clearance*

The protocol for animal experiments was approved by the Ethics Review Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka (No. 35/15).

*Animals*

Healthy adult female, Wistar rats weighing 150-200 g were purchased from Medical Research Institute, Colombo 8, Sri Lanka. Rats were housed under standard conditions with a natural light-dark cycle and fed with standard diet and water ad

libitum. The animals were acclimatized for at least one week to the laboratory conditions prior to the experiment.

*Preparation of 70% ethanol extract of A. pedunculate leaves (EEAL)*

One hundred grams of fresh leaves were refluxed with 500 mL of 70% ethanol for two hours. The extract was filtered and the filtrate was evaporated under reduced pressure to dryness. The residue was collected and dissolved in 0.5 % carboxymethyl cellulose (CMC) for oral administration to rats.

*Toxicity study of A. pedunculate leaves*

To evaluate the safety of the 70 % ethanol extract of *A. pedunculata* leaves, a limited dose acute oral toxicity study and sub-acute oral toxicity study (28 days) were carried out in compliance with the Organization for Economic Co-operation and Development (OECD) guidelines. In each assay, healthy control was administered with 1 mL of distilled water and the negative control group was administered with 1 mL of 0.5 % CMC. In the limited dose test, treated group received 5000 mg/kg b. w. of EEAL. The sub-acute toxicity study was done for the therapeutic effective dose of EEAL as found in anti-inflammatory assays (200 mg/kg b. w.) and doses which are lesser and higher than this dose, i.e.100 mg/kg b. w. and 2000 mg/kg b. w. respectively for 28 days. In each assay, assessment of mortality and the behavior of the animals were carried out by the general observations of each animal twice daily from the stage of dosing to the end of the study. Further, changes in the body weight, water and food consumption were compared with the control group. In addition to this haematological and biochemical parameters i.e. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), γ-glutamyltransferase (γ-GT), urea, creatinine, glucose, cholesterol, calcium and bilirubin levels, were measured to evaluate the safety of the plant extract. All the results were expressed as mean ± standard error of mean (SEM). Data was analyzed using one-way analysis of variance test (ANOVA) to determine the significance of the differences between the healthy control and test groups as well as in between the test groups. The $p$- values $< 0.05$ were considered as statistically significant. Further relative organ index was calculated to assess the safety of EEAL on different organs. Histopathological studies were also done.

**Results and Discussion**

During the entire period of all studies, there were no mortalities found following administration of EEAL. As mortality is the main criteria in assessing the acute toxicity of any drug, the absence of mortality by EEAL is an indicator of the safety. Further absence of any changes or abnormalities in the condition of fur, urine color, faeces or signs such as diarrhoea, salivation and breathing abnormalities, damaged skin, subcutaneous swelling or lumps, wetness or soiling of perineum in treated group in comparison to the control group indicated that EEAL in acute and sub-acute dosing is safe.

As the body weight is also an important factor to monitor the health of an animal, the OECD guidelines of toxicity testing place considerable emphasis on reporting on changes in weight gain of each animal. Loss of body weight is frequently the first indicator of the onset of an adverse effect. A dose which causes 10 % or more reduction in the body weight, is considered to be a toxic dose [2]. As EEAL treated groups in each assay have not shown the body weight reduction throughout the study periods, it provides the evidence for safety of usage of EEAL. Further, the results showed that, there were no significant ($p > 0.05$) difference in food and water consumption in treated groups as compared to negative control as well as in between three test groups.

When considering the serum clinical profiles, all tested parameters in the rats treated with EEAL comparable with those of the control rats of each study. The results on sub-chronic study are given in Table 1. As AST, ALT, ALP and γ- GT are good enzymatic indicators of hepatic diseases, the absence of any significant difference ($p > 0.05$) of those markers in EEAL treated group indicates lack of toxicity on liver. Further, absence of any significant difference ($p > 0.05$) in serum urea and creatinine levels in EEAL treated rat group as compared to the control group indicate lack of toxicity on kidney. The absence of any difference in relative weights of vital organs in treated as compared to the control group also provides scientific evidence for the safety of the test extract.

In addition to clinical chemistry profile, haematological parameters are also good indices of physiological and pathological status in humans as well as the animals. Hence, haematological parameters were also measured and any level of abnormal haematological parameters was not found in EEAL treated groups. The results on sub-chronic study are given in Table 2.

As histological assessment is also important in toxicology studies, the collected tissues were subjected for histopathology observations including leukocyte infiltration, haemorrhages in kidneys, necrosis in liver and several other features in each toxicity study. The results have shown that there were no any morphological changes in microscopic examination of tissues. Hence, it also provides strong evidence for the non-toxicity of EEAL.

**Table 1.** Clinical chemistry data of Wistar rats in the sub-acute oral toxicity study of *A. pedunculate* leaves

| Clinical chemistry parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Albumin (mg/dL) | 4.3 ± 0.1 | 4.4 ± 0.1 | 4.3 ± 0.1 | 4.3 ± 0.1 | 4.4 ± 0.1 |
| ALP (IU/L) | 117 ± 3.9 | 116.5± 3.6 | 116.9 ± 3.2 | 122.2 ± 3.5 | 116.7 ± 2.6 |
| ALT (IU/L) | 59 ± 10.4 | 43.6 ± 0.8 | 46.7 ± 2.4 | 48.3 ± 2.7 | 46.0 ± 2.2 |
| AST (IU/L) | 75.2 ± 2.2 | 72.0 ± 2.4 | 76.6 ± 3.1 | 77.8 ± 2.3 | 75.0 ± 2.0 |
| Calcium (mg/dL) | 12.1 ± 0.5 | 11.6 ± 0.4 | 11.6 ± 0.3 | 11.6 ± 0.2 | 11.1 ± 0.4 |
| Cholesterol (mg/dL) | 72.6 ± 3.5 | 74.4 ± 3.1 | 73.0 ± 3.6 | 75.8 ± 2.4 | 71.1 ± 3.4 |
| Creatinine (mg/dL) | 0.7 ± 0.05 | 0.6 ± 0.01 | 0.7 ± 0.02 | 0.7 ± 0.05 | 0.6 ± 0.06 |
| γ – GT (IU/ L) | 24.0 ± 1.1 | 23.8 ± 0.6 | 22.4 ± 1.1 | 22.8 ± 0.7 | 22.2 ± 1.7 |
| Glucose (mg/dL) | 84.0 ± 1.5 | 87.0 ± 1.1 | 80.1 ± 2.4 | 81.7 ± 1.2 | 83.2 ± 1.1 |
| Phosphorous(mg/dL) | 21.1 ± 2.3 | 19.5 ± 2.3 | 18.7 ± 2.4 | 19.6 ± 0.5 | 18.1 ± 0.7 |
| Total bilirubin (mg/dL) | 2.8 ± 0.3 | 3.0 ± 0.2 | 3.0 ± 0.2 | 3.5 ± 0.2 | 3.3 ± 0.4 |
| Urea (mg/dL) | 4.1 ± 0.4 | 3.6 ± 0.2 | 4.4 ± 0.3 | 3.2 ± 0.1 | 3.6 ± 0.3 |

Values for clinical chemistry parameters are expressed as mean ± SEM

Group 1: Healthy control group (DW), Group 2: Negative control group (0.5% w/v CMC), Group 3: Treated group (100 mg/kg b. w., EEAL in 0.5% w/v CMC), Group 4: Treated group (200 mg/kg b. w., EEAL in 0.5% w/v CMC), Group 5: Treated r group (2000 mg/kg b. w., EEAL in 0.5% w/v CMC)

**Table 2.** Haematological parameters of Wistar rats in the sub-acute oral toxicity study of *A. pedunculate* leaves

| parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Haemoglobin (g/dL) | 15.0 ± 0.4 | 15.0 ± 0.3 | 15.5 ± 0.5 | 15.4 ± 0.2 | 15.1 ± 0.4 |
| RBC (x $10^9$ / L) | 7.7 ± 0.2 | 7.8 ± 0.2 | 8.0 ± 0.2 | 8.1 ± 0.2 | 8.0 ± 0.3 |
| PCV (%) | 43.7 ± 0.9 | 43.6 ± 0.8 | 44.7 ± 1.4 | 44.5 ± 0.7 | 44.2 ± 1.3 |
| Platelet count (x$10^9$/L) | 847 ± 67 | 831 ± 67 | 883 ± 27 | 866 ± 60 | 840 ± 74 |
| MCV (fL) | 114 ± 0.8 | 95.8 ± 1.7 | 111.7 ± 1.9 | 92.7 ± 1.7 | 111.5 ± 1.6 |
| MCH (pg) | 38.9 ± 0.3 | 38.6 ± 0.1 | 38.7 ± 0.7 | 38.1 ± 0.6 | 38.1 ± 0.7 |
| MCHC (g/ dL) | 68.9 ± 0.7 | 68.9 ± 0.4 | 69.6 ± 0.5 | 69.4 ± 0.6 | 68.5 ± 0.3 |
| WBC ( x $10^9$ / L) | 8.5 ± 0.6 | 8.4 ± 0.5 | 7.0 ± 0.4 | 7.6 ± 0.6 | 7.5 ± 0.3 |
| Granules ( x $10^9$ / L) | 0.6 ± 0.1 | 0.7 ± 0.1 | 0.8 ± 0.3 | 0.6 ± 0.1 | 0.8 ± 0.1 |
| Lymphocytes(x$10^9$/ L) | 7.1 ± 0.2 | 6.9 ± 0.4 | 5.6 ± 0.5 | 6.2 ± 0.5 | 5.9 ± 0.2 |
| Monocytes( x $10^9$ / L) | 0.8 ± 0.2 | 0.9 ± 0.1 | 0.6 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 |

Values for haematological parameters are expressed as mean ± SEM, (n=6/group)

Group 1: Healthy control group (DW), Group 2: Negative control group (0.5% w/v CMC), Group 3: Treated group (100 mg/kg b. w., EEAL in 0.5% w/v CMC), Group 4: Treated group (200 mg/kg b. w., EEAL in 0.5% w/v CMC), Group 5: Treated r group (2000 mg/kg b. w., EEAL in 0.5% w/v CMC)

## Conclusion

The results of this study when considered along with the ethnomedical usage of *Achronia pedunculata* and its reported anti-inflammatory properties, suggests that the 70% ethanolic extract of the plant has the potential to be developed as a safe and effective treatment for chronic inflammatory conditions such as arthritis.

**Acknowledgement**

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# EVALUATION OF PHOTOPROTECTIVE POTENTIAL OF SUNSCREEN FORMULATIONS PREPARED FROM *Leucas zeylanica* (Gatathumba) EXTRACT

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## Introduction

The photoprotection is a term emerged in the modern-day science to alleviate the harmful effects caused by the UV radiation. This includes using of various photoprotective agents to reduce or avoid the detrimental effects of UV radiation. Topical application of sunscreens is the most prominent strategy to avoid the penetration of UV radiation into the skin. These sunscreen products contain active molecules which can absorb, reflect or scatter solar UV radiation. And these active molecules are mostly synthetic, which might cause adverse side effects such as allergies, hypersensitivity and oxidative damage to the skin. On the other hand the plant extracts have low side-effect profiles. Moreover Sri Lanka has a history of using indigenous medicinal plants for dermatological therapeutics as well as to improve skin complexion [1]. Therefore, it is worthwhile to assess the photoprotective capability of these plants in a systematic study. In our previous wok, we studied the photoprotective potential of aqueous methanolic extract of *Leucas zeylanica*. In this study, we explore the photoprotective potential of sunscreen formulations prepared from a methanolic extract of the whole *L. zeylanica* plant.

## Materials and Methods

*Preparation of crude extract*

The whole plants of *L. zeylanica* were collected, thoroughly washed and dried in shade for one week. The plant materials were authenticated by comparing the specimens at the National Herbarium, Royal Botanical Gardens Peradeniya, Sri Lanka and a voucher specimen (SG-2018-LZ-02) was deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka. Then dried plant material was powdered using a domestic grinder. The powdered plant material (10-15g) was subsequently extracted using 300 mL of methanol, and the extract was evaporated into complete dryness in a rotary evaporator and the residue was kept at a hot air oven for 2 days at 40 °C.

*Preparation of creams*

Different concentrations of the extract (i.e. 25%, 50% and 75%) were incorporated into an aqueous cream base to produce three sunscreen products.

*Determination of UV absorbance*

Each product was dissolved in distilled water to a concentration of 1mg/mL and then UV absorbance of the solution was measured from 260 nm to 400 nm using UV-Visible spectrophotometer.

*Determination of sun protection factor (SPF) and photostability*

The SPF value indicates the degree of photoprotection. We exposed the samples to direct sunlight for three consecutive weeks (6 hours per day)and the UV absorbance was measured. We used Mansur equation [2] to calculate the SPF after each week of exposure.

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

The SPF value of the formulation will be determined at 3, 6 and 9 months after its preparation to evaluate its stability. A commercial sunscreen product was used as the positive control and, the aqueous cream base without the extract was used as the negative control, in the experiment. The experiment was performed in triplicate and the results were presented as mean± S.D.

**Results and Discussion**

Table 1 presents the SPF values obtained from this study. The 75% formulation shows highest SPF values across the board, from the outset to the end of three weeks. This observation underlines the fact that, the higher the concentration of extract, the smaller the reduction of SPF value upon its exposure to sunlight.

**Table 1**. SPF values of different formulations in a span of three weeks

| Extract percentage (%) | SPF Values | | | |
|---|---|---|---|---|
| | **Initial** | **7 days** | **14 days** | **21 days** |
| **25** | 16.32 ± 0.05 | 13.67 ± 0.47 | 12.48 ± 0.35 | 10.91 ± 0.57 |
| **50** | 23.55 ± 0.04 | 20.03 ± 0.46 | 17.68 ± 1.08 | 14.15 ± 1.31 |
| **75** | 26.53 ± 0.08 | 24.42 ± 0.14 | 21.83 ± 1.51 | 18.66 ± 1.41 |

*Data represented as mean ± SD (n=3)*

Hence the 75% formulation possesses the highest photostability profile among all samples. At the same time, its SPF covers the whole UV-B range and most of the UV-A range.

Interestingly, the aqueous cream base which was used as the negative control did not display any UV-absorptive potential while the SPF value of the commercial sunscreen product used as the positive control was observed as 3.87±0.15. The commercial sunscreen product also displayed photostability.

**Conclusions and Recommendations**

According to the results it can be concluded that the formulation with 75% of the extract has the highest photoprotective activity. Furthermore, incorporation of a higher concentration of the extract into the sunscreen product is always beneficial to keep the product's activity in a regular state. These findings revealed that a methanolic extract of whole plants of *L. zeylanica* is containing secondary metabolites which can absorb UV radiation and those metabolites needs to be identified using phytochemical screening techniques.

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# VARIATION OF PHYTOCHEMICAL PROFILE OF *Cinnamomum zeylanicum* ACCORDING TO DIFFERENT ENVIRONMENTAL CONDITIONS

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## Introduction

Ceylon cinnamon (true cinnamon), scientifically known as *Cinnamomum zeylanicum* Blume belongs to the family Lauraceae. This 10-15m tall evergreen tropical plant is indigenous to Sri Lanka and Sothern part of India. The genus *Cinnamomum* contains more than 250 species found in tropical rain forest all around the world and Cinnamomum cassia (*Cinnamomum aromaticum* Ness) is the other main variety of cinnamon which is grow in China, Indonesia and Vietnam. Almost every part of the Cinnamomum tree including inner bark, leaves and root bark used as a main and popular spice and flavoring agent, not only for culinary uses but also for Ayurvedic medicines from the ancient period. In traditional medicine, cinnamon is used for respiratory, digestive and gynecological ailments and anti- clotting, anti-microbial, boosting of brain functions throughout the history. Cinnamon contains essential oils like cinnamic acid, cinnamaldehyde and cinnamate. Although in Sri Lanka phytochemical analysis of essential oil extracts in cinnamon bark and leaves has been reported adequately phytochemical profile for leaves and crude extracts not fully investigated. The aim of this study is to investigate of Ceylon cinnamon for their biochemical composition in leaves and bark at five different cinnamon cultivated districts of Sri Lanka.

## Materials and Methods

Cinnamon leaves and barks samples collected from different district received though Cinnamon research station, Palolpitiya, Matara in Sri Lanka were used in the study. Samples were washed and dried for seven days in dark place at room temperature. Finely grounded cinnamon leaves and bark samples were stored separately in dark colored screwed cap glass bottles at room temperature till used.

*Preparation of extracts for the analysis*

The methanolic extract of each plant sample is prepared by macerating 50 g of powdered samples in 200 ml of methanol for three days. The extracts are then filtered using Whatman No.1 filter paper

*Qualitative determination of phytochemicals constituents of cinnamon*

The methanolic extracts of the Ground leaves and bark samples were subjected to different phytochemical screening for alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, phlobatanins and glycosides using standard procedures. [1, 2]

*Quantitative determination of phytochemicals constituents of cinnamon*

*Determination of Alkaloids*

50 mL of 10% acetic acid in ethanol was added to 0.5 g of sample and allowed to stand for 4h. The supernatant was filtered using Whatman No.01 filter papers and the extract was concentrated on a water bath to one-quarter of the original volume. 25% ammonium hydroxide was added to the hot extract until the precipitation completed. the precipitate was filtered using 0.45µm membrane filter papers and washed with 10mL 0f 0.1M ammonium hydroxide and dried in oven at 400C till constant weight obtained. [3, 4]

*Determination of Flavonoids*

12.5mL of 80% methanol was added to 0.5g of sample and allowed to sedimentation for 24 hours at room temperature. Supernatant was discarded and the residue was extracted tree times by adding 10mL of ethanol for a once. Using Whatman No.01 filter papers whole extract was filtered and collected to a crucible. Then the filtrate was evaporated on a water bath and remains were cooled in a desiccator until constant weight was obtained. [3, 4]

*Determination of Saponins*

0.5 g of sample was macerated for 4h adding 20% ethanol (25.0 mL) and filtered. This step repeated twice. Whole the extract was evaporated to one-half in a water bath at $90^0$C and vigorously agitated with diethyl ether (10.0 mL) in separatory funnel for twice. Diethyl ether layer was discarded and 25.0mL of n-Butanol was added to the extract and it was washed twice by 10mL of 5% sodium chloride. After sedimentation n-Butanol layer was collected to a crucible and evaporated in a water bath. The remains were dried in an oven temperature $40^o$C until constant weight was obtained. [3, 4]

*Determination of Oxalate*

1.0 g of sample was continuously stirred with o.3M hydrochloric acid (10.0 mL) at $50^0$C for 1h and filtered. To the 25% ammonium hydroxide (10.0 mL), two drops of phenolphthalein indicator, acetic acid (1.0 mL) and 5% calcium chloride (1.0 mL), 5mL of filtrate was added to it. After standing it was centrifuged at 3000 rpm for 15 minutes. The precipitate formed was washed with hot water and dissolve in 2.0mL of 5M sulphuric acid by warming in a water bath at $70^o$C. Then the mixture was titrated by freshly prepared 0.01M potassium permanganate until the first pink color appeared. [4]

*Determination of Phenols*

0.5g of sample was continuously stirred with 10mL of distilled water for 3 hours at room temperature. The mixture was filtered using Whatman No.01 filter papers and 1.0mL of extract was transferred into a 25.0mL volumetric flask.5mL of dyeing solution ( 1.0g ferrous sulfate and 5g sodium potassium tartrate dissolved in 1000mL distilled water) and 5mL of buffer ( 0.067M potassium phosphate, pH 6.5) was added to the flask and volume up with distilled water. The absorption at 540nm was measured after 30 minutes. [5]

*Determination of Lipids*

0.5g of sample was added 3mL of chloroform: methanol solution (1:2) and homogenized on a water bath for 1h. The mixture was centrifuged at 3000rpm for 15 minutes and supernatant was added 2mL of 1% potassium chloride and vertex well. The mixture was centrifuged at 3000rpm for 5 minutes and the lower layer was transferred into a crucible. After the lipid mixture was evaporated at room temperature the remains were stored in a desiccator until constant weight obtained. [4]

**Results and Discussion**

The results carried out on the qualitative phyto-constituent of *C. zeylanicum* leaf and bark samples are presented in Table1. In these screening process alkaloids, tannins, saponins, flavonoids and terpenoids, glycosides and Phlobatannins shows same results in different district. Saponins and Phlobatannins content of bark are higher than leaf. The data shows that strong positive results for alkaloids, flavonoids, tannins and terpenoids in leaf methanolic extracts and alkaloids, flavonoids, tannins, saponins, terpenoids and Phlobatannins strongly positive in methanolic extract of cinnamon bark.

**Table1.** Qualitative phytochemical screening of leaf and bark successive extracts of *Cinnamomum zeylanicum*.

| Sl No. | Phyto-constituent | Leaf | Bark |
|---|---|---|---|
| **1** | Alkaloids | ++ | ++ |
| **2** | Flavonoids | ++ | ++ |
| **3** | Tannins | ++ | ++ |
| **4** | Saponins | + | ++ |
| **5** | Terpenoids | ++ | ++ |
| **6** | Steroids | + | + |
| **7** | Phlobatannins | + | ++ |
| **8** | Glycosides | + | + |

*+ Positive results, ++ strong positive results.*

The quantitative analyzed of phyto-constituent for cinnamon leaves and bark extracts are given Table 2 and Table 3 respectively.

**Table 2.** Content of Phytochemicals in Cinnamon leaves samples according to the districts.

| | District | | | | |
|---|---|---|---|---|---|
| **Sample** | **Galle(mg/ 100 g FW)** | **Matara(mg / 100 g FW)** | **Hambanthota( mg/ 100 g FW)** | **Kandy(mg / 100 g FW)** | **Kurunegala(mg / 100 g FW)** |
| Alkaloids | 3.63±0.12 | 0.96±0.18 | 3.45±0.03 | 3.15±0.12 | 3.53±0.18 |
| Flavonoids | 4.36±0.08 | 0.91±0.12 | 3.38±0.41 | 2.82±0.73 | 3.36±0.29 |
| Saponins | 6.78±0.04 | 5.55±0.20 | 5.90±0.20 | 7.67±0.75 | 7.46±0.05 |
| Phenol | 4.76±0.02 | 0.52±0.12 | 1.81±0.76 | 3.86±0.10 | 1.18±0.30 |
| Lipid | 5.48±0.20 | 1.44±0.35 | 3.99±0.25 | 5.60±0.70 | 7.68±0.03 |
| Oxalate | 2.78±0.09 | 1.26±0.03 | 3.71±0.49 | 3.89±0.21 | 3.92±0.24 |

*Values represent mean ± standard deviation of triplicate sample.*

The highest polyphenol, flavonoid content while hare important as anti-oxidant in Galle district cultivars and the highest lipid and oxalate content in the Kurunegala district cultivars. Kandy district cultivars have the highest saponins content.

**Table3.** Content of Phytochemicals in Cinnamon bark samples according to the districts.

| | District | | | | |
|---|---|---|---|---|---|
| **Sample** | **Galle(mg/ 100 g FW)** | **Matara(mg / 100 g FW)** | **Hambanthota( mg/ 100 g FW)** | **Kandy(mg / 100 g FW)** | **Kurunegala(mg / 100 g FW)** |
| Alkaloids | 6.43±0.04 | 4.02±0.08 | 6.19±0.36 | 5.39±0.10 | 3.55±0.28 |
| Flavonoids | 2.15±0.09 | 1.67±0.06 | 1.92±0.47 | 1.96±0.40 | 1.56±0.09 |
| Saponins | 9.54±0.14 | 8.83±0.10 | 13.49±0.20 | 7.67±0.75 | 8.22±0.25 |
| Phenol | 13.40±0.32 | 25.48±0.30 | 18.21±0.93 | 4.10±0.13 | 15.52±0.39 |
| Lipid | 3.38±0.20 | 1.36±0.10 | 1.47±0.22 | 1.56±0.20 | 2.23±0.13 |
| Oxalate | 2.87±0.19 | 2.83±0.13 | 3.24±0.39 | 4.07±0.41 | 3.53±0.16 |

*Values represent mean ± standard deviation of triplicate sample.*

The highest polyphenols, flavonoids and alkaloids content (mg/100g) in leaves were 4.76±0.02 4.36±0.08 and 3.63±0.12 respectively from the cultivars in Galle district whereas the highest flavonoids and alkaloids content (mg/100g) in bark were 2.15±0.09 and 6.43±0.04 respectively for the same cultivars in Galle district, contrast to the leaves, the highest polyphenols content in the bark was found in the cultivars in Matara district which is 25.48±0.30 mg/100g. The lowest alkaloid content was found in leaves as 0.96±0.18 mg/100g for the cultivars in Matara district whereas 3.55±0.28 mg/100g was recorded for the bark from Kurunegala district.

**Conclusions and Recommendation**

This finding show that both leaf and bark extracts of *C. zeylanicum* contains important phytochemicals. When compare with leaf extracts, the bark extract has high availability of those. The content of the phytochemicals has dependency on the environment or geographical factors. When compare all, the important phytochemical content especially flavonoids and polyphenols are good in the cultivars in Galle and Matara district. The lowest alkaloid content was reported as 0.96±0.18 mg/100g for the cultivars in Matara district is another good factor.

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# *IN-SILICO* INVESTIGATION OF INHIBITORY EFFICACY OF SELECTED HYDROXAMIC ACID DERIVATIVES ON HISTONE DEACETYLASE ENZYME

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## Introduction

Involvement of Histone Deacetylase (HDAC) enzymes in cancer development is associated with certain oncogenes and tumor suppressor genes both are major players in the molecular pathways leading to human cancer. In many cancers HDAC enzymes have gone into overdrive, stripping off acetyl groups from crucial DNA region those regulate the activation of tumor suppressor genes.

Computational chemistry approaches have made significant advances in identifying molecules, which are potent inhibitors of deacetylation activity of HDAC enzymes. This study is a preliminary investigation on using computational techniques to compare the effect of hydroxamic acid derivatives on HDAC enzyme. This was accomplished by computing the positional stability of individual amino acids in terms of the surrounding environment of the cofactor of the HDAC enzyme. It is hoped that this may lead to the development of a molecular-level description of the inhibition efficacy of an inhibitor. Ramachandran plot and LigPlot$^+$ were used to analyze data obtained from molecular dynamics studies to investigate and compare the inhibition efficacy of Panobinostat and Reminostat with the reference drug Suberoylanilide Hydroxamic Acid (SAHA).

Ramachandran plot is formed by considering the atoms as simple imaginary spheres. The three major regions in a Ramachandran plot are favored, allowed and statically disallowed are shown in Figure 1.

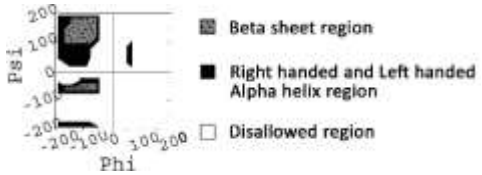



**Figure 1.** Model of Ramachandran plot, indicating the three regions indifferent colors and symbol

The regions with + symbol correspond to conformations, where there are no steric clashes, in other words, these are the allowed regions namely the alpha-helical and beta-sheet conformations.

The black areas show the allowed regions where slightly shorter van der Walls radii are used for the atoms to come little closer together. This region brings additional areas, which correspond to the left-handed alpha-helixeswhich are mirror images of those in the alpha-region.

The white areas correspond to conformations, where atoms in the polypeptide come closer than the sum of their van der Waals radii. These regions are sterically disallowed for all amino acids except glycine and proline. LigPlot$^{+}$ diagram (Figure 4) gives a schematic depiction of each inhibitor molecule. This displays hydrogen bonds and non-bonded interactions between the inhibitor and the amino acids of the protein.

**Methodology**

X-ray crystal structure of Histone Deacetylase (PDB ID 1ZZ1) was downloaded from the protein data bank [1]. The optimized structures of three inhibitor compounds (derivatives of hydroxamic acids) are shown in Figure 2which were generated in Gaussian 09 and each structure was converted to the PDB format using Avogadro software.

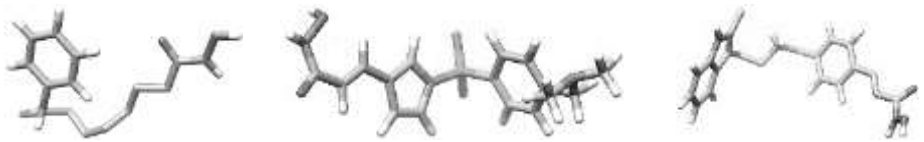



**Figure 2.** The three HDAC inhibitors used in this study; (a) Suberoylanilidehydroxamic acid (Reference inhibitor drug) (b) Reminostat (c) Panobinostat

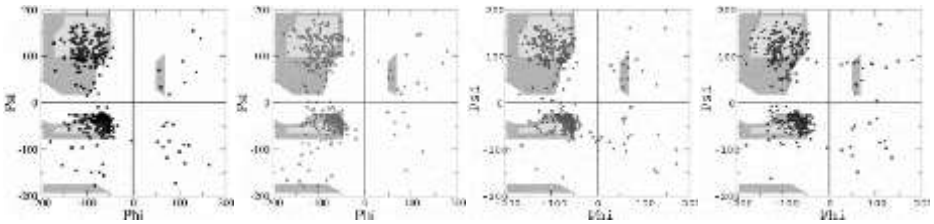
AutoDock Tools 1.5.6 docking software package, as implemented in Vina was used to select the best binding scores of HDAC-inhibitor complexes. These complexes were used as the starting configurations for molecular dynamics (MD) studies. In the MD simulation, GROMOS53a6 force field was employed for the enzyme and PRODRG server was used to generate the force field parameters for the drugs [2, 3]. The enzyme-drug complex was inserted into the center of an 8.8 x 8.8 x 8.8 $nm^3$ cubical box. Then the cubical box was filled with 20,000 SPC/E water molecules to solvate the complex and 11 $Na^+$ ions were added to maintain electrical neutrality of the system.

Particle mesh Ewald (PME) with a short-range cut-off of 1.2 nm was used to model the electrostatic interactions [4]. Temperature and pressure of the system were modulated at 300 K and 1 bar respectively using Berendsen's weak coupling algorithm [5]. LINCS algorithm was used to constrain all bonds at their equilibrium distances.

Similar simulation conditions were employed for the enzyme without inhibitor (wild type enzyme) to study the effect of the inhibitor on the enzyme. All the systems were subjected to 500 steps of energy minimization with the steepest descent algorithm followed by 100 ps molecular dynamics simulation to equilibrate the system. After the equilibration step, molecular dynamic simulations were performed for 100 ns on all enzyme-drug systems studied and the wild type enzyme with a 0.002 ps time step using GROMACS molecular dynamics simulation package running in LINUX operating system. The trajectories of the outcomes were saved at every picosecond for further analysis. Properties of the Panobinostat and Reminostat were compared with those of Suberoylanilidehydroxamic acid (SAHA) since it is the well-known inhibitor drug in clinical practice.

**Results and Discussion**

Molecular dynamics trajectory files were analyzed to study the structural stability of residues of the enzyme with simulation time. The Ramachandran plot and LigPlot+ were prepared for each inhibitor-HDAC complex and HDAC. Ramachandran plots of the enzyme, in each enzyme-drug complex were compared with others, and the comparison is shown in Figure 3.



(a) (b) (c) (d)

**Figure 3.** Ramachandran plots of enzyme residues. (a) Wild type enzyme(b) SAHAcomplex (c) Reminostat complex (d) Panobinostat complex

As seen in Figure 3 many of the amino acid residues fall into three major clusters closely associated with the alpha-helical, beta-sheet and left-handed alpha-helix regions. Some angles also fall into disallowed regions.

**Table 1.** List of amino acids that interact with inhibitors at different stages

| Complex | Before MD simulation | After MD simulation |
|---|---|---|
| **HDLP / SAHA** | LEU21,ASP98, **HIS143,ASP180,HIS182,**PHE208, **ASP268,TYR312,Zn370** | ASP98, GLY99, ILE100, **HIS142,HIS143,** MET150, **ASP180,HIS182**, PHE208, ASP268, TYR312, **Zn370** |
| **HDLP/ Reminostat** | ILE100, **HIS143,** PHE152, ASP180, HIS182, ASP268, **TYR312**, **Zn370** | HIS142, HIS143, PRO145, MET150, CYS153, ASP180, ASP182, PHE208, ASP268, **TYR312**, **Zn370** |
| **HDLP / Panobinostat** | ILE100, **HIS142,** HIS143, **ASP180, HIS182,** PHE208, **ASP268,** LEU275, **TYR312, Zn370** | LEU21, ASP98, ILE100, **HIS142, HIS143,** PRO145, MET150, GLY151, ASP180, HIS182, PHE208, PRO209, ASP268, LEU275, **TYR312, Zn370** |

** The bold phase letters indicate that the amino acid is directly bonded to drug through hydrogen bond.*

The Ramachandran plot clearly shows that the number of residues in disallowed region is low for both the reference inhibitor SAHA and new inhibitor Panobinostat. When compared with the stability of the wild type enzyme, these two inhibitor drugs bring the HDAC to a more stable state.

Panobinostat stabilizes all the residues, which are unstable in wild type enzyme. Most of the glycine residues fall into the disallowed region because glycine has only a hydrogen as a side chain and has very little steric hindrance as phi ($\phi$) and psi ($\psi$) angles are rotated through a series of values. So, glycine is an exception to Ramachandran plot.

The surrounding environment of the drug and the Zn (II) ion is analyzed through LigPlot$^+$ software. The results of LigPlot$^+$ are shown in Figure 4.

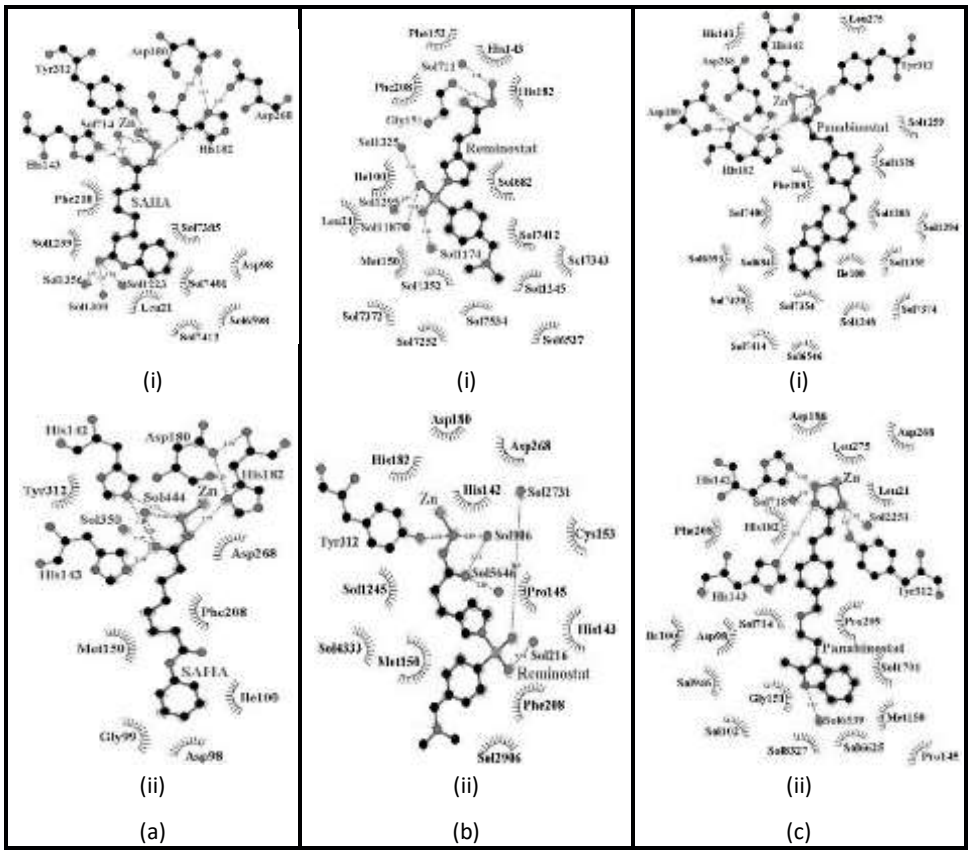



**Figure 4.** Surrounding environment of drug and Zinc co-factor. (a) SAHA complex, (b) Reminostat complex, (c) Panobinostat complex. (i) Before MD simulation, (ii) After MD simulation.

Before MD simulation, many water (solvent) molecules were present in the surrounding region of the inhibitor and Zn (II) ion; however, after the simulation, some of those water molecules were replaced by amino acid residues. So, it is clear that during the MD simulation, many amino acids develop interactions such as hydrogen bond with the inhibitor and come closer, leading to increased stability. Detailed analysis of Ramachandran plot shows that LEU21, HIS142 and GLY151 are present in disallowed region in wild type enzyme that moved to the allowed region when bonded to Panobinostat. The results of LigPlot$^{+}$ also strongly justify this observation. During the simulation, HIS142 is directly interacting with Panobinostat via a hydrogen bond. The other amino acids are also attracted by the inhibitor and result hydrophobic interaction with the inhibitor drug. By this process, these amino acids become stable during MD simulation. Similarly, HIS142 forms a hydrogen bond and directly interacts with SAHA, where GLY99 shows hydrophobic interaction with SAHA. Interaction with SAHA moves these residues to the stable region in the

Ramachandran plot. The inhibitor Reminostat also brings the residue HIS142 to the stable region of the Ramachandran plot.

**Conclusion and Recommendation**

This *in-silico* study demonstrates that, under the stabilization function, the new inhibitor, Panobinostat exerts a similar effect on the enzyme as the reference drug, SAHA.It is evident that Panobinostat has the potential to be used as an alternative to the reference drug SAHA, for the inhibition of histone deacetylation. In contrast that Reminostat shows some similarities, as well as many deviations from SAHA and Panobinostat that makes Reminostat relatively less efficient since both Panobinostat and Reminostat are in clinical trial III. The $IC_{50}$ values of Panobinostat and Reminostat have been reported as 5 nM and 42 nM respectively. The result of this *in-silico* study also reflects the above trend.

**Acknowledgment**

National Science Foundation is greatly acknowledged for providing funds for this study (Project number RG/2018/BS/02).

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# INVESTIGATION OF THE IMPACT OF Cd (II) ION ON HUMAN URACIL DNA GLYCOSYLASE: AN *IN-SILICO* APPROACH

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## Introduction

Non-communicable diseases have become a major problem for low and middle income countries including Sri Lanka. The enzyme human Uracil DNA Glycosylase (hUNG) is recognized as a major enzyme that associates with non-communicable diseases like cancer since it can be affected mostly by heavy metals due to their toxicity in the ionized form like Cd (II), As (III), As (V). hUNG, a DNA repair enzyme has rendered possible for the initial detection due to the removal of carcinogenic lesions from DNA. Research studies have found that Cd (II) ion, is involved in the process of inhibiting the activity of the enzyme, hUNG. Experimental works report that, the removal of catalytic water of hUNG by Cd (II) ion and the formation of a stable metal complex, as the reasons for preventing the catalytic process of the enzyme and inhibiting the enzyme's activity [1].However, major problem is the lack of sufficient research work to understand the role of metal ions in the inhibition of the enzymes. Hence, this work can be considered as a preliminary work done using computational techniques to study and explain mechanistically the Cd (II) mediated inhibition of the enzyme hUNG causing non-communicable diseases.

## Materials and Methods

The crystal structure of the enzyme hUNG (PDB ID: 1AKZ) and Cd (II) ion that is, one among the most toxic and highly abundant heavy metal ions were downloaded from the protein data bank. The binding cavities, the corresponding residues in the cavities and the ligandability which is the ability of binding a ligand to a binding site in a cavity were determined using the Cavityplus server by uploading the pdb coordinates of the enzyme to the server [2]. Then, the MIB server [3] was used to predict the metal ion binding sites of the enzyme with Cd(II) ion.

A molecular dynamics (MDs) simulation was carried out for the system consists of hUNG enzyme and Cd(II) ion with Kirkwood-Buff force field.[4] The system was placed in a cubical box where the minimum distance between the protein and the edge of the box was 1.5 nm. To solvate the system 20620 SPC/E water molecules were required while seven (07) chloride ions were added to maintain the electro-neutrality of the system. After 3000 steps of energy minimization a

short MD run was carried out at 1 bar pressure and 300 K temperature for 10 ps. Then a 50 ns long MDs simulation was conducted at constant NPT conditions. The pressure was maintained at 1 bar with Parrinello-Rahman barostat method and the temperature was maintained at 300 K with Nose-Hoover thermostat. The same protocol was used for a 50 ns long MDs simulation of the enzyme alone, where 20649 SPC/E water molecules were required to solvate the enzyme while five (05) chloride ions were added to maintain the electroneutrality of the simulation system. For both simulations, the configurations were saved at every 01 ps intervals for further analysis.

**Results and Discussions**

The number of cavities with the geometrical shape of the binding sites of the enzyme and the relevant residues in the cavities were determined using the CavityPlus server. Seven cavities were identified for the enzyme and they are shown in Figure 1.

**Figure 1**: Geometrical shape of the seven cavities obtained for the enzyme hUNG.

The Server represents the ligandability by a value and the values less than 6.0 are considered as not a suitable binding site for the ligand. Table 1 gives the ligandability of the cavities of the enzyme.

**Table 1**. Ligandability of the cavities of the enzyme hUNG.

| Cavity number | Ligandability |
|---|---|
| **1** | 9.91 |
| **2** | 10.13 |
| **3** | 9.83 |
| **4** | 7.21 |
| **5** | 6.50 |
| **6** | 6.05 |
| **7** | 5.94 |

According to the ligandability in Table 1, the binding of a ligand to the binding site of cavity number 7 is less favourable compared to the remaining six cavities. Hence, the cavity number 7 can be considered as being a not suitable binding site with respect to the CavityPlus server results. The MIB server was used to predict the metal ion binding sites of the enzyme (Figure 2) with predicted scores to represent binding potentials of Cd(II) ion (Table 2).

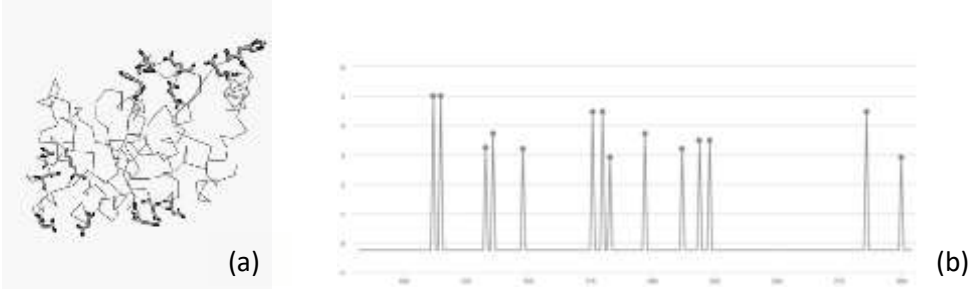


**Figure 2**. MIB server results for the enzyme hUNG with the Cd (II) ion. (a)Metal ion binding residues are shown as sticks in the protein image. (b) Predicted scores as the binding potential for the residues of the enzyme hUNG.

**Table 2.** Binding residues of the enzyme with the predicted scores as binding potentials of Cd (II).

| Residue number | Residue | Predicted Score |
|---|---|---|
| **112** | GLU | 4.999 |
| **115** | HIS | 4.999 |
| **133** | ASP | 3.239 |
| **136** | ASP | 3.717 |
| **148** | HIS | 3.195 |
| **176** | GLU | 4.462 |
| **180** | ASP | 4.462 |
| **183** | ASP | 2.908 |
| **197** | LYS | 3.717 |
| **212** | HIS | 3.195 |
| **219** | GLU | 3.483 |
| **223** | GLU | 3.483 |
| **286** | LYS | 4.462 |
| **300** | ASP | 2.908 |

According to Figure 2 and the results in Table 2, the enzyme hUNG possesses 14 residues among the 223 residues that show a high binding score and hence, they can bind with the Cd (II) ion.

Geometrical properties of the enzyme were analyzed using Ramachandran plots. Figure 3 (a), (b), (c), (d) and (e) represent how the residues vary in the

Ramachandran plot at different simulation times and Figure 3 (f) shows the same in the absence of the metal ion.

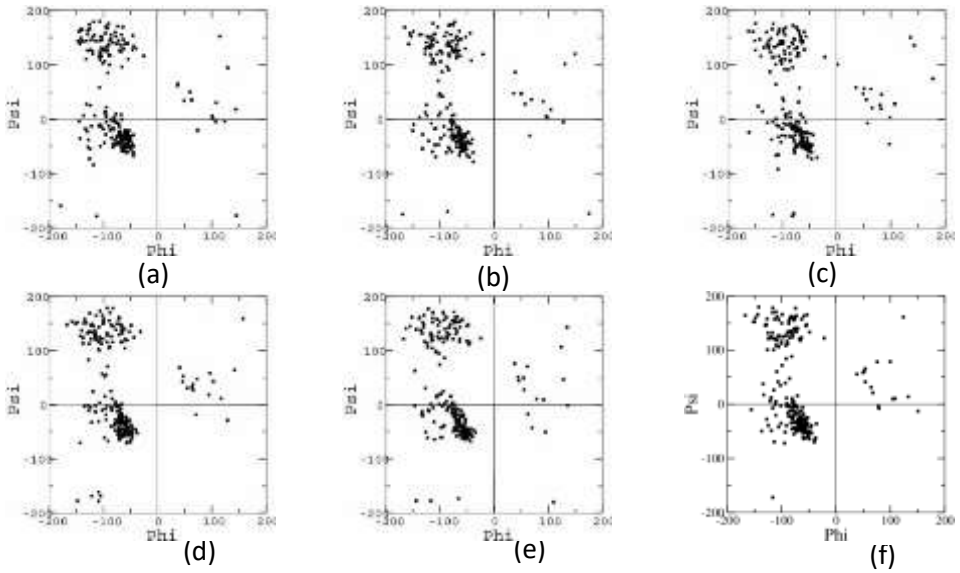


**Figure 3.** Variation of the residues in the regions of Ramachandran Plot. (a) after 10 ns (b) after 20 ns (c) after 30 ns (d) after 40 ns (e) after 50 ns (f) enzyme without the metal ion after 50 ns

The fourth quadrant of the Ramachandran plot is considered as the prohibited region and it is sterically forbidden for all amino acids with side chains. The diagrams (a), (b), (c), (d) and (e) of Figure 3, can be used to identify the stability of the enzyme when Cd(II) ion is in the medium by studying the variation of the residues in the regions of the plot at different simulation times. Since the metal ion goes around the enzyme by interacting with the residues during the simulation until it finds a stable confirmation, the number of residues in the prohibited region also varies and finally it comes to a minimum value. The diagram (f) of Figure 3 illustrates how the variations take place in the absence of the metal ion.

The root mean square deviation (RMSD), radius of gyration (Rg) and root mean square fluctuation (RMSF) in Figure 4 were obtained from the trajectory files after 50 ns simulation of the two systems where with and without Cd(II) ion in the medium.

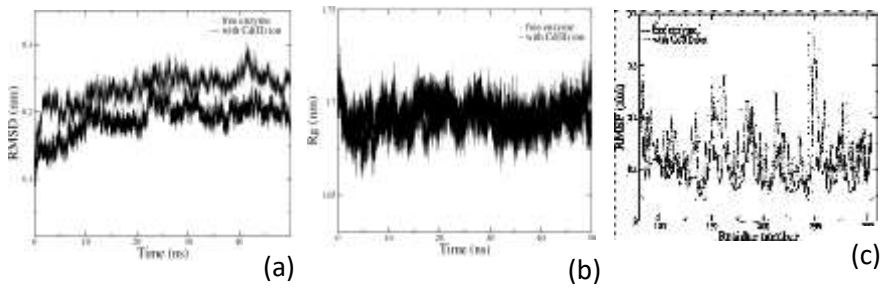


**Figure 4**. The structural behavior of the enzyme with and without the Cd (II) ion. (a) RMSD (b) Rg (c) RMSF

According to Figure 4(a), the RMSD of the enzyme in the two cases with and without the Cd (II) ion increases up to around 20 ns and then shows a less variation. However, the RMSD where Cd (II) ion is in the medium shows less deviation than the RMSD of the free enzyme. The radius of gyration (Figure 4(b)) indicates the compactness of the enzyme. The fluctuations of the residues can be observed from Figure 4(c). When comparing the two fluctuation curves, a high fluctuation of the residues in the free enzyme can be observed than in the enzyme with the ion.

**Conclusions and Recommendations**

The RMSD and Rg curves of the hUNG in the two cases with and without metal ion, interpret that both the cases have been stabilized during the 50 ns simulation. Hence, it can be concluded that there can be a stable confirmation of the enzyme hUNG with Cd (II). The fluctuations of the residues in the enzyme have been restricted when Cd (II) ion is in the medium. Hence, the enzyme with Cd (II) ion shows a less fluctuation behavior. And also, RMSD of the enzyme, when Cd (II) is in the medium, is less than the RMSD of the free enzyme. These results imply that there can be an effect on the enzyme hUNG by the Cd (II) ion. Simulations for the enzyme with the Cd (II) ion for longer trajectories and by placing the metal ion closer to the residues, that were identified as binding residues from MIB server, can be carried out further to identify the best binding site with binding energies. Further, it will be possible to identify whether those binding sites are indeed the cavities obtained from CavityPlus server.

**Acknowledgement**

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

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# SPECTROSCOPIC DETERMINATION OF CHEMICAL ELEMENTS ON ABUNDANTLY AVAILABLE FIVE LEAF TYPES IN TROPICS

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## Introduction

Plants are compelling biochemists' man is able to obtain from them a wondrous assortment of industrial chemicals. Both past and present farming cultures of the world have intentionally improved the soils on which they planted. In the past, people have practiced in utilizing natural treatment technics to overcome their challenges in agriculture. The use of plants parts for different purposes has been with man from the beginning mean time the technologies evolved. Studies of the chemistry of environmental enclosures provide critical evidence that can strengthen and augment interpretations based on its chemical analysis. Around 7000 B.C since mankind started cultivating, they had devised technics and practices on preventing the crops destroying by natural disasters as well as remarkably from insects. They were adopted selecting naturally resistant plants. Farming cultures have long understood that dung and organic debris may enhance or restore soil productivity. By detecting the ancient use of fertilizers which derive from historical periods can lead towards a rich source od data for modern industries [1]. Texts documenting the nature and application of fertilizer potentially shed light on many situations. In the absence of texts, it is possible to reconstruct these practices through stratigraphic, chemical, and botanical analysis. In turn, studies of natural fertilizers or the natural plant parts utilizes mainly the leaves can reveal aspects of past agricultural practices that is valuable for the local environment.

Scanning electron microscopy is discussed in light of its principles, advantages, and applications. This is closely related to the electron probe, is designed primarily for producing electron images, but can also be used for element mapping, and even point analysis, if an X-ray spectrometer is added. There is thus a considerable overlap in the functions of these instruments [2]. This was due partially to the ideal surface and structural makeup of wood that lends itself to this type of investigation [3]. Energy Dispersive X-Ray Spectroscopy (EDS or EDX) EDS makes use of the X-ray spectrum emitted by a solid sample bombarded with a focused beam of electrons to obtain a localized chemical analysis. All elements from atomic number 4 (Be) to 92 (U) can be detected in

this principle, though not all instruments are equipped for 'light' elements (Z < 10) [3-7].

## Materials and Methods

Aim of this study was to determine the atomic percentage of chemical elements and obtain information about the surface topography and composition of five types of air-dried leaves; which was identified as alternatives for pesticides used in crops to improve soil.

**Table 1.** Selected leaf types

| Sample No | Scientific name | Family name | Local name |
|---|---|---|---|
| S-1 | *Adhatoda vasica* | Acanthaceae | Agaladhara, Pawatta, Vanepala |
| S-2 | *Croton aromaticus* | Euphorbiaceae | Wel keppetiya |
| S-3 | *Tithonia diversifolia* | Compositae | Mexican sunflower, Walsooriyakantha |
| S-4 | *Azadirachta indica* | Meliaceae | Kohomba Leaves |
| S-5 | *Gliricidia sepium* | Fabaceae | Wetahiriya. Wetamara, Ladappa, Nanchi, Sevana, Kola Pohora |

Methodology - Several analytical techniques have been used effectively for solid state identification analysis of plant materials until today. In this particular study five types of Leaves were analyzed by using SEM-EDAS analysis. The chemical compositions of the samples will be investigated by energy dispersive X-ray analysis using EDX Z1 Analyzer to fit to ZEISS EVO/18 Research Scanning Electron microscope. The leaves were randomly picked from the plant from the same location: Kanupellala Badulla. Then washed from distilled water and kept for air drying for 4-5 days. Randomly selected three points were then considered to perform an area analysis.

## Results and Discussion

SEM involves scanning a focused beam of high-energy electrons over the surface of a sample in order to produce a variety of signals that inform us of certain characteristics of the sample, including topography (surface shape) and chemical composition.

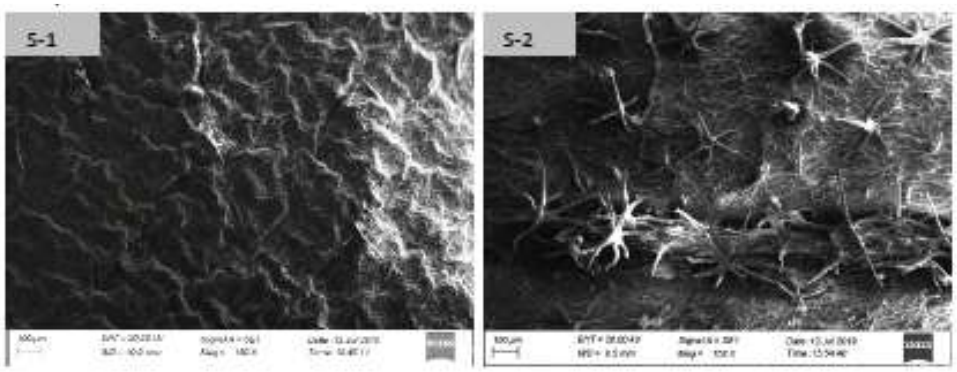


**Figure 1**. Scanning electron microscopic Images of the selected points of the leaf under 150 X magnification; (S-1; Pawatta leaves), (S-2; Wel keppetiya Leaves).

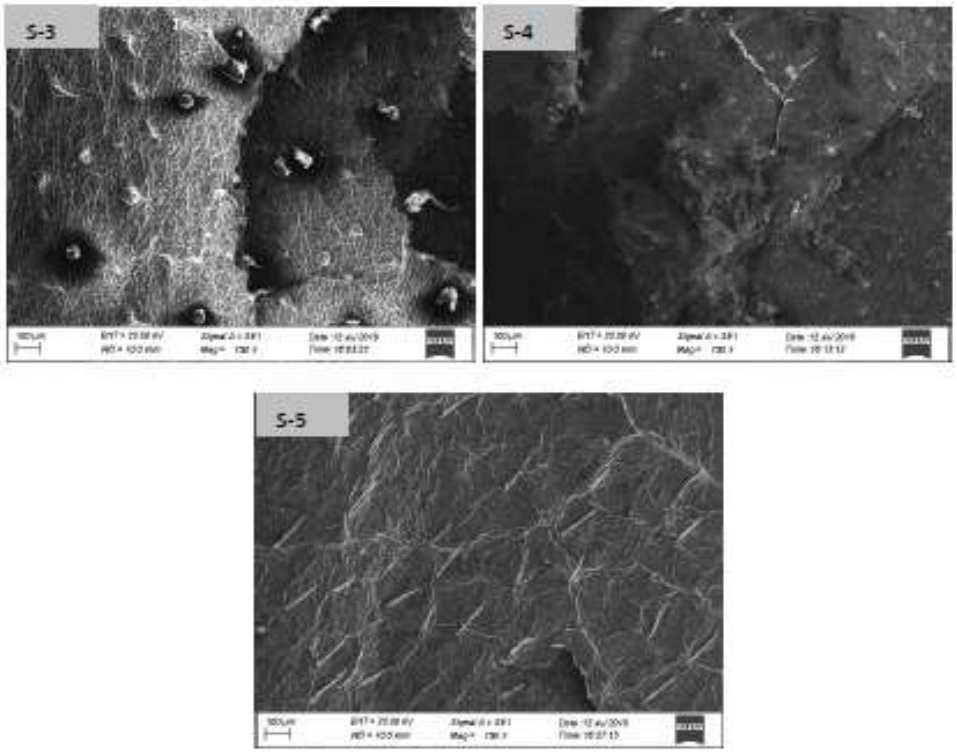


**Figure 2**. Scanning electron microscopic Images of the selected points of the leaf under 150 X magnification; (S-3; Walsooriyakantha leaves), (S-4; Kohomba Leaves), (S-5; Nanchi or Gliricidia)

Analyzed elements were as below

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CARBON | OXYGEN | MAGNESIUM | SILICON | PHROSPOROUS | CHLORINE | POTASSIUM | CALCIUM | ALUMINIUM | FERROUS | SULPHUR | SODIUM |
| C | O | Mg | Si | P | Cl | K | Ca | Al | Fe | S | Na |

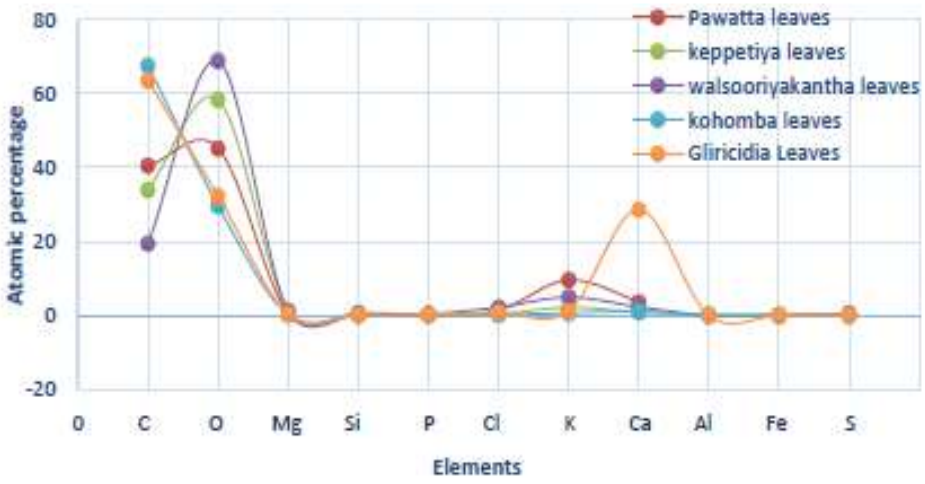


**Figure 3**. Atomic percentage, chemical elements present in air dried leaves including Carbon and Oxygen

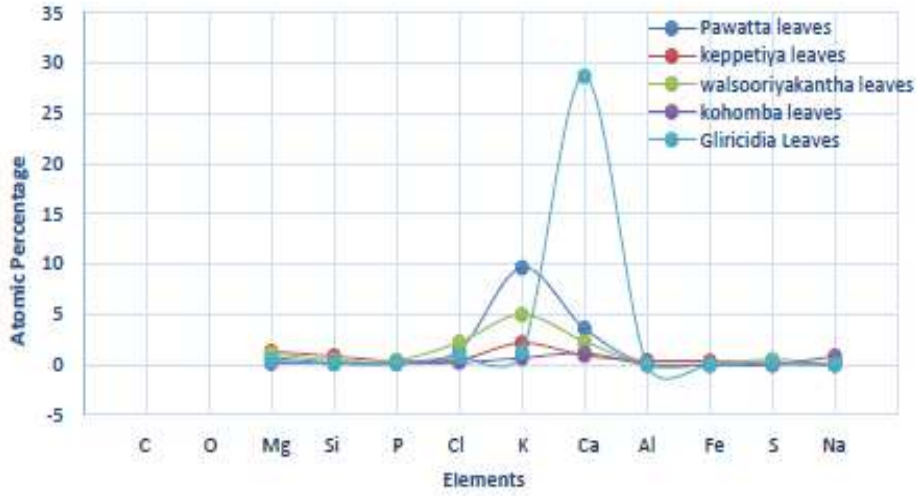


**Figure 4.** Chemical elements present in air dried leaves excluding Carbon and Oxygen

**Conclusions and Recommendations**

A rich heritage of knowledge on preventive was available in ancient knowledge and practice in Sri Lanka [8]. Among them a practice of utilizing natural technics were established among the farmers in Sri Lanka. They were utilizing leaves such Pawatta, Vanepala, Wel keppetiya, Walsooriyakantha, Kohomba, Wetahiriya. Wetamara, Ladappa, Nanchi, Sevana to improve their soil as a fertilizer as well as pesticides.

Most pesticides contain other elements, among them Chlorine, oxygen, sulfur, phosphorus, nitrogen, and bromine are most common. Inert ingredients can be many substances, dependent on the type of pesticide. Pesticides are by their toxic substances by nature. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds and so on, that is any part of the plant may contain active components. The elemental analysis on above mentioned five selected types of plants showcased that carbon, oxygen, magnesium, silicon, phosphorous, chlorine, potassium, calcium, aluminum, ferrous, Sulphur, sodium visibly available in different atomic percentages. The results highlighted majority of the leaves contained higher atomic percentages of potassium. Also, walsooriyakantha and keppetiya leaves contains considerably high atomic percentages of Sulphur which was not presented in other types. Ecotoxicology for Sulphur the element sulfur is used as in insecticides, and fungicides [9]. According to literature it is proved that Potassium comprises a pesticide type Microbicide [10]. It is clear our farmers might not learn the application through a theoretical knowledge. But the experienced knowledge had proved the potentials and the successes of utilizing such fertilizers together had incorporated pesticidal effects with lesser human risk.

Outcome and how the work will be applied**;** though world has introduced pesticides and developed with the concept of target organism toxicity, often non-target species as well as mankind are affected badly by their applications. The purpose of this review is to list some of the major utilized natural leaves as pesticides under the traditional practice in Sri Lanka, to understand the Atomic percentages of the chemical elements that are leading towards a role in playing as pesticides based on their activity and determination, and also to understand their biochemical toxicity.

**Acknowledgement**

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Engineering, Faculty of Engineering, University of Moratuwa, Sri Lanka for technical Support.

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# FEASIBILITY OF INCORPORATING BIYAGAMA DRINKING WATER TREATMENT PLANT SOLID SLUDGE IN A CONSTRUCTION MATERIAL PRODUCTION PROCESS

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## Introduction

Water is one of the basic needs of human, as well as a main requirement of all living creatures. Access to sufficient drinking water amount is important for human in physiologically as well as psychologically [1]. Therefore secure the access to safe and sufficient drinking water amount of the residences is one of the main responsibilities of the country; to secure the public health. Sri Lanka is a country, rich with high quality natural water resources compared to the other countries in the world. But with the urbanization and industrialization; access to safe water is restricted in most of the urban areas in present. Therefore the Sri Lankan government has to provide sufficient and safe drinking water to the residences in urban areas of the country. National water supply and drainage board (NWSDB) is responsible in the drinking water supply of the country [2]. NWSDB provide its service along with 323 major, medium and small water supply schemes operated all over the country.

In the water supply schemes, water from surface water reservoirs were collected and treated to drinking quality through water treatment plants to remove impurities. Drinking water treatment plants (DWTP) consist with processes of coagulation, flocculation, sedimentation, filtration and disinfection [3]. In the coagulation process chemical coagulants; such as alum ($Al(SO_4)_2$), lime ($Ca(OH)_2$) and polymers, were added to remove colloidal and suspended impurities such as sand, silt, clay and humic particles. Flocculation and sedimentation processes let the sludge to settle and removed in the filtration process. The sludge is an inevitable waste product generate in DWTP.

Biyagama DWTP collects raw water from Kelani River and produces 180,000$m^3$ of drinking water per day, which covers 1,000,000 of population. The plant has a sludge treatment plant to dewater the sludge and to remove solid sludge. The sludge treatment plant is consist with two wash water recovery tanks, a sludge balancing tank, a sludge thickener tank, a thickener sludge tank and two sludge decanters. In the sludge treatment plant water is recovered from sludge and feed back to the DWTP. The dewatered solid sludge is generated in the rate of 10$m^3$ per day in the Biagama drinking water treatment plant.

DWTP sludge is categorized under nonhazardous industrial waste[4]. Although it is nontoxic, discharge to water bodies is prohibited due to the undesirable formation of mud deposits which creates potential disturbances to the aquatic biota and the water reservoirs. Biyagama DWTP solid sludge sent to fill rock blasting wells and land fillings but with the time solid sludge generation of the plant is increasing and a sustainable solution should be found.

DWTP sludge was identified as a fine material made with organic and suspended matter, coagulant products, microorganisms and chemical elements and similar to mud in texture. In the construction industry mud can be identified as an effective construction material from the prehistoric time. Mud is use as a construction material in brick production; mud concrete block and cement stabilized earth block, roof tile production, wall construction; rammed earth walls and mud concrete walls. Therefore the DWTP sludge can be used as an alternative raw material to mud to use as a construction material after identifying the properties of the sludge.

The properties of the sludge is depend on the water treatment process, sludge treatment process, raw water quality, treated water quality, climatic conditions and etc. Therefore DWTP sludge of each plant consists with different intrinsic properties [5]. Therefore the effective and sustainable solution for the increasing generation of DWTP dewatered solid sludge is to incorporate in a construction material production process. According to the variation of intrinsic properties of DWTP sludge, properties of the Biyagama DWTP sludge should be identified first. Therefore this study was conducted to identify the feasibility of incorporating Biyagama DWTP dewatered solid sludge in a construction material production processs in Sri Lanka by identifying the properties of the sludge.

**Materials and Methods**

Dewatered solid sludge sample was collected from the sludge treatment plant of the Biyagama DWTP. To compare the properties of the DWTP sludge with the properties of pure mud; mud sample was prepared by mixing sieved soil clay partices with water. To analyze the physical properties of the sludge sample and mud sample, laboratory experiments were conducted to measure the moisture content (Oven dry method), solid content (ASTM C1603-16) and volumetric shrinkage (ASTM D4943-18). In the each test, three replicates from two samples (sludge and mud) were tested and the average value was calculated.

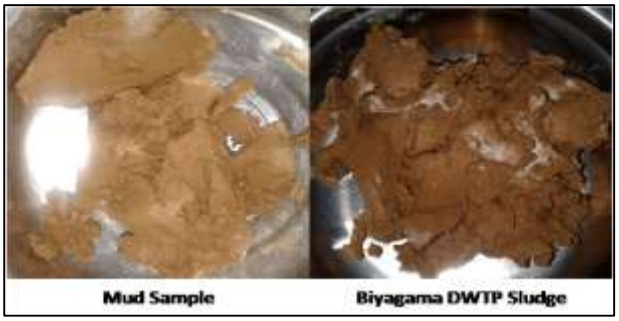


**Figure 1**. Biyagama DWTP sludge and mud samples

*Physical properties test*

Moisture content and the solid content of samples, in weight basis were tested according to the oven dry method. The wet weight (WW) of each sample was measured and the samples were oven dried in $110^0$C temperature. After 24 hours the dry weight (DW) of each sample were measured. Moisture content (MC) and the solid content (SC) of each sample were calculated according to the equation 1 and 2, respectively.

$$MC(\%) = \frac{Ww - Dw}{Ww} \quad \text{.....(1)}$$

$$SC(\%) = 100 - MC \quad \text{.....(2)}$$

Volumetric shrinkage of each sample was measured considering the difference of initial volume and volume after drying. Samples were cast in 70x70x70 $mm^3$ moulds and unmolded after 7 days and let to dry. Three dimensional measurements were taken weekly until 35 days; where the drying is completed. Volumetric shrinkage (VS) of each mixture was calculated according to equation 3; where *V1* is initial volume ($70^3$ $mm^3$) and *V2* is final volume at the measuring date.

$$VS(\%) = \frac{V1 - V2}{V1} \quad \text{.....(3)}$$

**Results and Discussion**

*Physical properties test results*

Moisture content and solid content test results are shown in figure 2. Biyagama DWTP sludge consists with 32% solid and 68% of moisture. Mud sample consists with 81% solid content and 19% of moisture. According to the test results Biyagama DWTP sludge is higher in moisture content and lower in solid content compared to the mud sample. According to the literature the recommended moisture content of mud was in between 18-20%, to use as a construction material. Therefore the moisture content of the Biyagama DWTP sludge sample is not in the required range to directly use as a construction material.

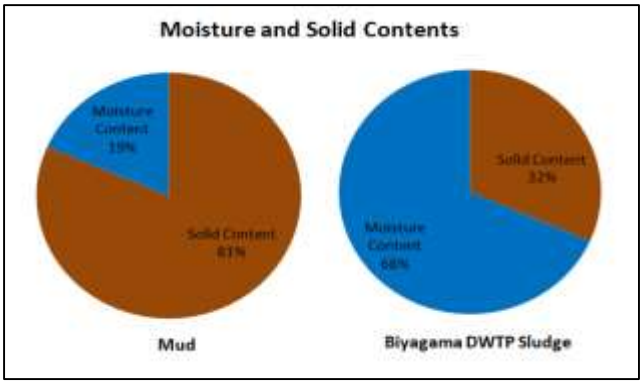


**Figure 2.** Moisture content and solid content of Biyagama DWTP sludge and mud

Volumetric shrinkage test results are shown in figure 3. According to the test results, the shrinkage of Biyagama DWTP sludge was calculated as 75.76% after 35 days and the shrinkage of mud was calculated as 29.4% after 35 days. Therefore shrinkage of Biyagama DWTP sludge is much higher, compared to the shrinkage of mud. The low solid content and high moisture content of Biyagama DWTP sludge results the more volumetric shrinkage compared to mud.

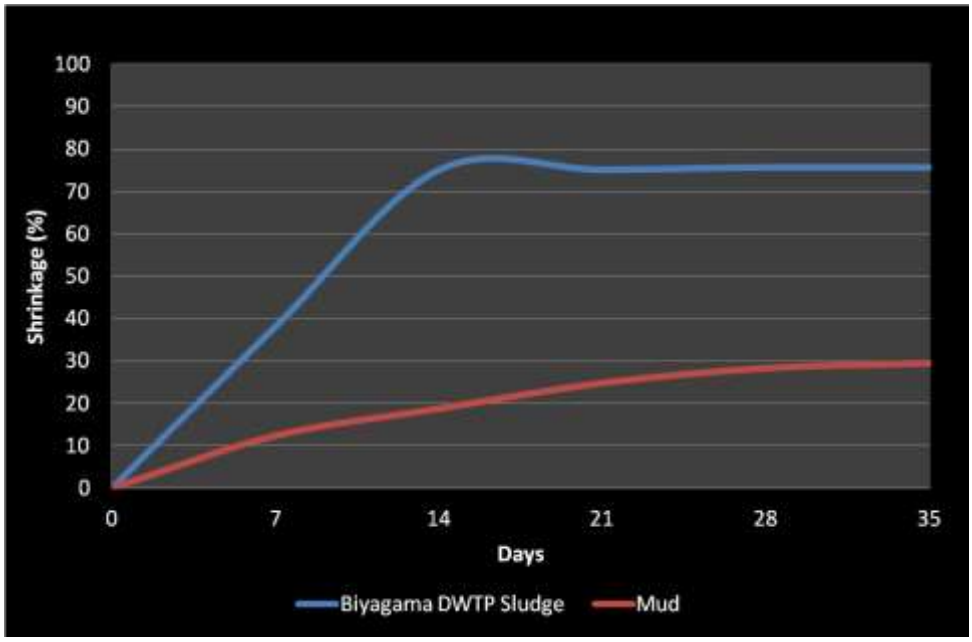


**Figure 3.** Volumetric shrinkage variation of Biyagama DWTP sludge and mud

## Conclusions and Recommendations

Although the Biyagama DWTP sludge is similar to mud in texture, there are some differences in physical properties. As the Biyagama DWTP sludge consists with higher moisture and low solid content compared to the recommended

values (moisture content in between 18-20) for mud as a construction material; volumetric shrinkage is significantly higher than mud. Therefore the dewatered sludge of the Biyagama DWTP cannot be directly utilized as a construction material as natural mud.

Further treatments and improvements should be done to reduce the moisture content and to reduce the volumetric shrinkage of the sludge to use as a construction material. Therefore further studies should be conducted to identify the possible treatment methods and technologies to improve the properties of Biyagama DWTP sludge to use as a construction material in Sri Lanka.

**Acknowledgement**

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# PHYTOCHEMICALS PRESENT IN LEAVES OF LOCALLY GROWN GUAWA VARIETIES; *Psidium guajava* (COMMON GUAVA), *Psidium cattleianum* (STRAWBERRY GUAVA) AND *Psidium guineense* (EMBUL GUAVA) UNDER DIFFERENT EXTRACTION TECHNIQUE

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## Introduction

*Psidium guajava* L. (Myrtaceae), popularly known as guava, is originally from Central and South America and has been cultivated in all tropical and subtropical countries. The literature has revealed that leaves of guava are rich with potential phytochemicals and are encompassed with various pharmacological activities namely anti-microbial activity, antidiarrheal activity, hypoglycemic activity, etc. [1,2]. Most of the studies on guava leaves has been reported in other countries and very less studies are available in Sri Lanka [3]. As Sri Lanka is a hotspot for diverse medicinal plants including different guawa varieties, investigation of Sri Lankan guawa varieties will lead to find potential compounds and potential healthcare applications through converting them into different functional foods and nutraceuticals. Of many guawa varieties available in Sri Lanka, four main varieties were considered in this study which includes common guava with middle size fruit (*P. guajava*), common guava with small size fruit (*P. guajava*), strawberry guava (*P. cattleianum*) and embul guava (*P. guineense)*. As conventional extraction methods require many hours to be performed, accelerated extraction methods were taken into consideration. The aim of this study was to identify the phytochemical classes present in the leaves of above four varieties of guava under two different extraction conditions, mainly maceration and sonication.

## Materials and Methods

*Sample collection*

Fresh and healthy leaves of guava verities were plucked from home gardens around Matara area and those were authenticated. The leaves were plucked from 3-4 plants of particular variety. After cleaning followed by open-air drying, it was grinded into powder to be used in the extraction process.

*Extraction*

A part of dried powder of each variety was extracted using maceration with methanol for 48 h at room temperature whereas the other part was extracted using ultrasound assisted extraction method (ROCKER Ultrasonic cleaner, Model: SONER 202H) at room temperature with methanol for one hour. The

extracts were filtered through cotton plug and the solvent was evaporated under vacuum at 45 $^0$C using a rotary evaporator.

*Phytochemical Screening*

Screening tests for phytochemicals were carried out as triplicated for the methanolic extracts of leaves of each variety, by following the standard procedures described in the literature [4, 5]. Under this, crude extracts were tested for glycosides, flavonoids, alkaloids, tannin, carbohydrates, proteins, amino acids, polyphenols, saponins, terpenoids, anthocyanins, Phlobatannins, coumarin, phytosterol, betacyanin, chalcones and quinones.

## Results and Discussion

The extracts of leaves of *P. guajava*, *P. cattleianum* and *P. guineense* have revealed the presence or absence of phytochemicals and the corresponding results are tabulated below.

**Table 1.** Results of triplicated qualitative analysis for leaves extract of *P. guajava*, *P. cattleianum* and *P. guineense* using maceration and sonication (1-4 maceration & 5-8 sonication).

| **Name of Phyto-chemicals** | **Extraction type** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Maceration** | | | | | **Sonication** | | |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| Alkaloids | P | P | P | P | P | P | P | P |
| Glycosides | P | P | P | P | P | P | P | P |
| Flavonoids | P | P | P | P | P | P | P | P |
| Saponins | P | P | P | P | P | P | P | P |
| Tannins | P | P | P | P | P | P | P | P |
| Terpenoids | P | P | P | P | P | P | P | P |
| Carbohydrate | P | P | P | P | P | P | P | P |
| Phenols | P | P | P | P | P | P | P | P |
| Phlobatannins | A | A | A | A | A | A | A | A |
| Protein | P | P | P | P | P | P | P | P |
| Amino acid | A | A | A | A | A | A | A | A |
| Coumarins | P | P | P | P | P | P | P | P |
| Anthocyanins | A | A | A | A | A | A | A | A |
| Chalcones | A | A | A | A | A | A | A | A |
| Phytosterol | P | P | P | P | P | P | A | P |
| Betacyanin | A | A | A | A | A | A | A | A |
| Quinones | P | P | A | P | P | P | A | P |

($^P$: Present, $^A$: Absent, $^{1,5}$: Common guava (Middle), $^{2,6}$: Common guava (Small), $^{3,7}$: Strawberry guava, $^{4,8}$: Ambul guava)

The study showed the presence of highly important secondary metabolites, phytochemicals, in the leaves of all four guava varieties. Out of them, alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids, carbohydrates, phenol, proteins, coumarins, phytosterol and quinones are remarkable in the leaves of *P. guajava*, *P. cattleianum* and *P. guineense* under two different extraction methods (exception – quinones absent in *P. cattleianum*). Interestingly other specific secondary metabolites such as phlobatanins, anthocyanins, chalcones

and betacyanin were absent in methanolic extracts of *P. guajava*, *P. cattleianum* and *P. guineense*. Phytosterol was absent in sonicated *P. cattleianum* (strawberry guava). This result supports the outstanding pharmacological activities associated with guava, which have been reported by various researchers and also the applications of guawa in traditional medicine as phytochemicals are known to be responsible for the biological activities exerted by plants. The results have shown that the extraction techniques of maceration and sonication have no significant effect on phytochemicals extracted from leaves. Though all the above guawa varieties are not very popular among the people, these results proves that those varieties can be equally used to secure the fruits requirements and the researcher can use those varieties for the value adding purpose.

**Conclusion**

The leaves of *P. guajava*, *P. cattleianum* and *P. guineense* contain vast array of potential phytochemicals. There is no significant difference in phytochemical profile of leaves among guawa varieties studied. Therefore, all varieties have equal potential for development into functional food as the extension of this study. Moreover, as there is no significant difference on phytochemical profile when extraction is done under maceration and sonication, accelerated extraction method through sonication would be recommended for extraction of phytochemicals efficiently.

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# ARIMA MODELS OVER DENGUE CASES REPORTED IN COLOMBO AND JAKARTA

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## Introduction

Dengue is a virul infection which is growing and spreading rapidly in the entire world. The World Health Organization found that around 390 million dengue cases reported annually [1]. The attention of the public on dengue disease has increased due the increasing number of infected people with the dengue. Effective and efficient modelling of the dengue will be able to reduce the burden of the disease. Under this aim the objective of this study is to model the reported dengue cases particularly in Colombo, Sri Lanka and Jakarta, Indonesia using AutoRegressive Integrated Moving Average (ARIMA) technique. The Colombo is the commercial capital of Sri Lanka whereas the Jakarta is the capital and the largest city of Indonesia. The selection of these two cities was based on the data availability. The monthly reported dengue cases in Colombo and Jakarta from January 2010 to November 2017 used for the study. The most suitable model among several possible ARIMA models to predict dengue cases in each of the two cities were found by based on AIC, AICc and BIC measures. Availability of an effective prediction model will helpful in anticipating the dengue and to make timely actions on controlling the dengue incidence.

## Materials and Methods

The following tests and methods were used in the study.

*Augmented Dickey Fuller (ADF) Test 01*

This test is capable in detecting whether a series has a unit root or not. The test statistics for the model $Y_t = \rho Y_{t-1} + u_t$ is $\frac{\hat{\rho}}{SE(\hat{\rho})} \sim t_{n-1}$ where $-1 < \rho < 1, u_t$ is the white noise, *n* is the number of observations. Null hypothesis is series is non-stationary.

*Kwiatkowski–Phillips–Schmidt–Shin (KPSS) test 02*

The null hypothesis of this test is an observable time series is stationary around a deterministic trend against the alternative of a unit root. This test is based on a linear regression. It breaks up a series into three parts: a deterministic trend ($\beta_t$), a random walk ($r_t$), and a stationary error ($\varepsilon_t$), with the regression equation: $x_t = r_t + \beta_t + \varepsilon_t$. If the data is stationary, it will have a

fixed element for an intercept or the series will be stationary around a fixed level.

*Autocorrelation Function (ACF) and Partial Autocorrelation Function (PACF) 03*

The coefficient of correlation between two values in a time series is represent the autocorrelation function. For example the ACF for a time series $x_t$ is given by:

$$\mathrm{Corr}(x_t, x_{t-k}), k = 1,2,\ldots.$$

This value of $k$ is the time gap being considered and is called the lag.

The partial autocorrelation function (PACF) is a summary of the relationship between an observation in a time series with observations at prior time steps with the relationships of intervening observations removed. The PACF plot can be used to identify the order of autoregressive (AR) process; that is the $p^{th}$ order of ARIMA (p, d, q) model. The $q^{th}$ order will be identified through ACF plot [4].

*Autoregressive Integrated Moving Average (ARIMA) 04*

ARIMA is a univariate time series model uses to capture the seasonal and other patterns in the data in order to forecast the future values of the series. There are basically three stages in the ARIMA modelling; Model identification, Parameter estimation and Model diagnostic checking. After seasonality and stationarity has being identified autoregressive and moving average components will be identified using ACF and PACF plots. In model diagnostic checking residuals should follow a white noice which is drawn from a constant mean and variance. If the assumptions are not hold then another model need to be investigated otherwise model can be used to make predictions [2].

*Seasonal Autoregressive Integrated Moving Average (SARIMA) 05*

SARIMA models can be used to model range of seasonal data. There are two parts in the SARIMA model formulation; one is for non-seasonal and the other is for seasonal. The seasonal part of an autoregressive (AR) and/or moving average (MA) model identified from the seasonal lags of the PACF and ACF. The modelling procedure is almost the same as for ARIMA procedure.

*Model Selection Criteria 06*

In the situation of two or more models fit with the available data in an acceptable framework then model selection criterions can be used to select the best model. Some of the widely used model selection criterions are; Akaike Information Criteria (AIC), Bayesian Information Criteria (BIC) and Corrected Akaike Information Criteria (AICc).

**Results and Discussion**

R software is mainly used for the analysis [3]. Figure 1 shows the time series plot of the reported dengue cases over the Colombo and Jakarta from January 2010

to November 2017. The highest number of dengue cases reported in July 2017 in Colombo and that is for Jakarta is in April 2016. Otherthan those two peak values dengue cases ranges in between 0 and 4000.

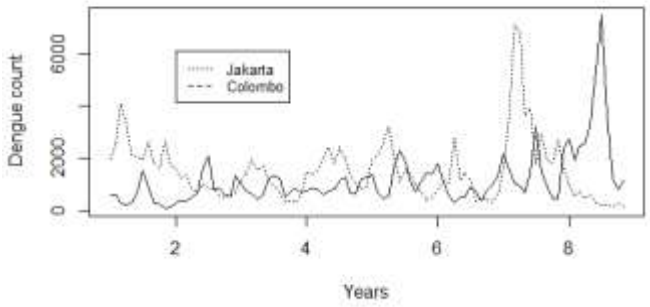


**Figure 1.** Reported dengue cases in Colombo and Jakarta from 2010-2017

Data from January 2010 to December 2015 used for model development and rest of the data for model validation. According to the ADF and KPSS tests there were non-stationarities in the original series of Colombo and Jakarta at 5% significance level and those overcome by taking first differencing. Then the stationarity of differenced series confirmed by ADF and KPSS tests at 5% significance level. The ACF and PACF plots were obtained for both series and significant lags were identified through the plots for both seasonal and non-seasonal AR and MA terms in SARIMA model. By changing various parameters of autoregressive and moving average components of both seasonal and non-seasonal parts of SARIMA model, optimum models for each city were found which have minimum AIC, BIC and AICc measures. Then optimum models were check for validity of assumptions. Residual analysis of both models represent in Figure 2 and Figure 3. All the assumptions of residuals satisfied by optimum models whereas Ljung-Box test is not significant at 5%. The selected best model for Colombo is ARIMA (1,1,2) $(1,0,0)_{12}$ and that is for Jakarta is ARIMA (0,1,0) $(1,0,0)_{12}$. The percentage errors of validation results from January 2016 to November 2017 were less than 20%. Therefore, the selected two models were used to forecast dengue cases from January 2016 to June 2018 in each of the city. Forecasted values are shown in Figure 4 and Figure 5 with 80% and 95% confidence intervals.

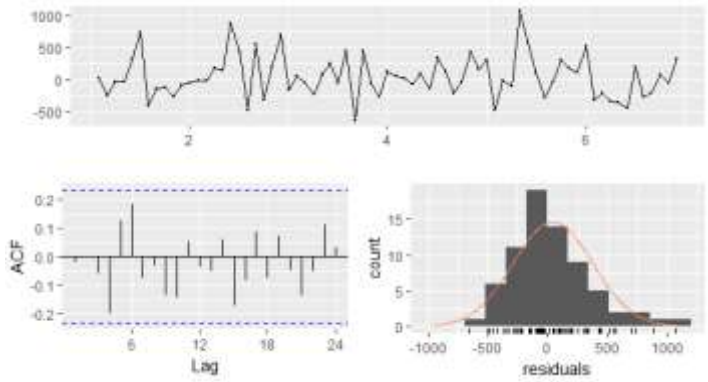


**Figure 2.** Residual analysis of ARIMA (1,1,2) $(1,0,0)_{12}$

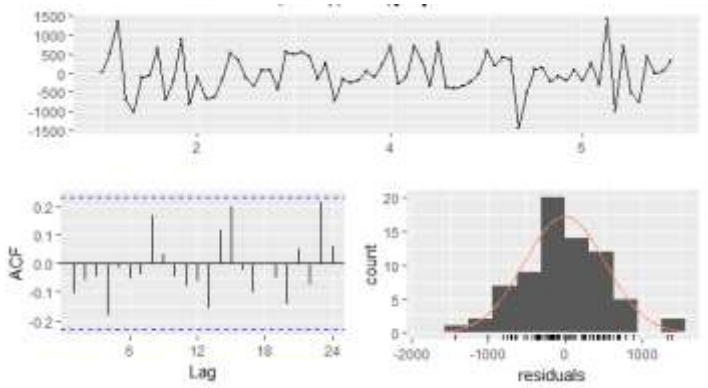


**Figure 3.** Residual analysis of ARIMA (0,1,0) $(1,0,0)_{12}$

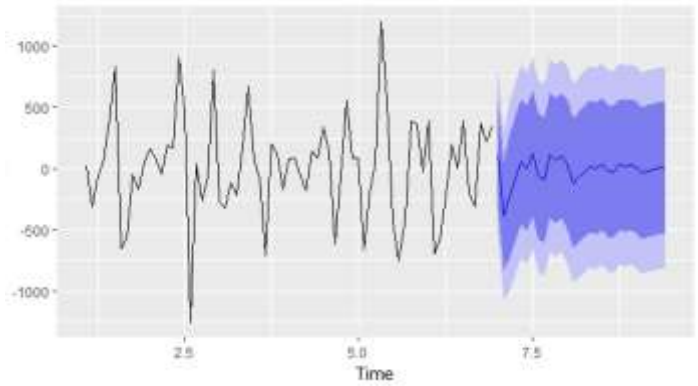


**Figure 4.** Forecasts from ARIMA (1,1,2) $(1,0,0)_{12}$

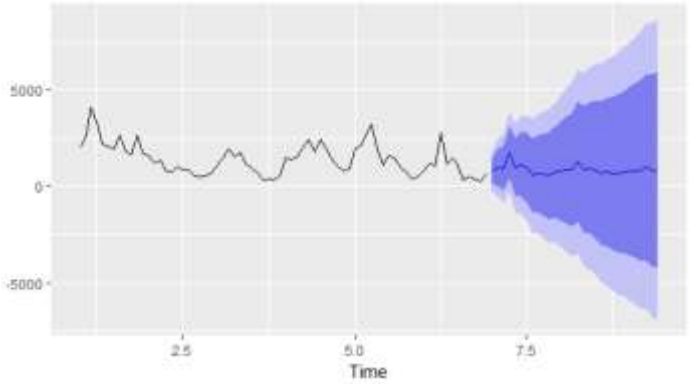


**Figure 5.** Forecasts from ARIMA (0,1,0) $(1,0,0)_{12}$

Even though the forecasts generated from best ARIMA models for each city there was a large difference of the forecasted values with the actual reported dengue cases from January 2016 to November 2017 in both cities. There were peaks of actual reported dengue cases in 2017 in Colombo data and in 2016 in Jakarta data. Hence, ARIMA models itself are not suitable in predicting dengue incidence because sudden changes of reported dengue cases cannot be captured by past values of the series. Therefore, ARIMA models are inappropriate in forecasting dengue incidence in Colombo as well as in Jakarta. One possible way to overcome the lack of fits of the models is to model dengue cases with associated other external factors such as climatic factors (Temperature, rainfall, etc.) as well as with non-climatic factors (human mobility, garbage collection patterns, etc.).

**Conclusions and Recommendations**

This study successfully models the monthly reported dengue cases in Colombo and Jakarta through ARIMA modelling technique with the aim of forecasting future dengue cases. The best model to predict monthly dengue cases in Colombo selected as ARIMA (1,1,2) $(1,0,0)_{12}$ and that is for Jakarta is ARIMA (0,1,0) $(1,0,0)_{12}$. However, the predictions of best models had differences with actual figures. All of the significant external factors that can be associated with dengue distribution should be identified through a comprehensive study and those factors should be included into the model development in order to make better predictions.

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# COMPARISON OF FORECASTING METHODS OF HEDGE RATIOS FOR STOCK MARKET INDEX PRICES IN SRI LANKA

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## Introduction

Risk is an essential yet unpredictable element of investing. Due to the volatility in stock market, investing money in stock is often considered as riskier compared to the less volatile assets, since the return is expected to be less predictable. Hedging is defined as a risk trading exercised in financial markets. Individual investors use hedging techniques to reduce their exposure to investment risks. In this study we focus on determining an effective way of predicting hedge ratios between stock, gold, exchange rates and treasury bonds with maturity years of six, in order to model an optimal investment portfolio forecasting method. Due to the fluctuations in the stock market, investors may have to face with the losses in their investments in stocks. Nevertheless, a well-diversified portfolio will assist to minimize the risk as risk is diversified. Objective of the study is to forecast a set of hedge ratios that provides the most effective hedge for stock market fluctuations under certain assumptions.

In our study, we consider the fluctuations in the stock market illustrated by S&P SL20 index in order to identify the stock market behavior. As shown in figure 1, stock market has diversified fluctuations in the considered time period of 27th June, 2012 to 31st August, 2018. Due to this reason, an investment in stock market is associated with high risk. Gold prices (figure 3) tend to indicate short term fluctuations and in the considered time period, an upward trend is observable. Contrast to the Sri Lankan economy, US Dollar rate (figure 2) is appreciating, thus, investing in US Dollar itself would give a chance to earn higher returns. When considering the treasury bonds (figure 4), we expect a lesser fluctuation in market quote values but in Sri Lankan context, significant fluctuations in secondary market quotes percentages can be observed. Nevertheless, it is a standard asset since it consists a government guaranteed repayment. Thereby in our hedging mechanism, gold prices, exchange rates and treasury bonds are considered as hedging assets to compensate any loss from investment in stock market. Two methods are used to figure out the most effective method to forecast a set of hedge ratios in order to effectively hedge the stock market fluctuations under certain assumptions

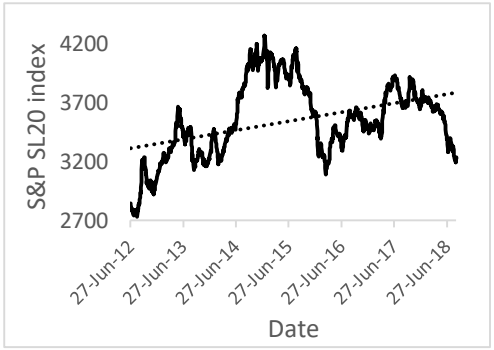


**Figure 1.** Fluctuations in S & P SL 20 index

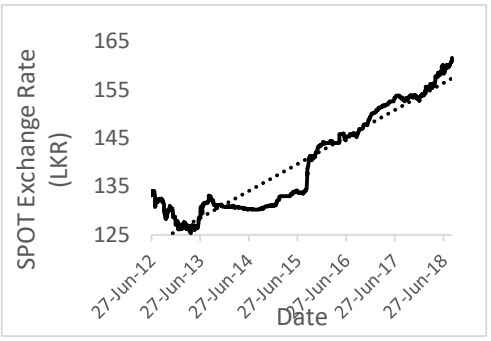


**Figure 2.** Fluctuations in SPOT Exchange Rates

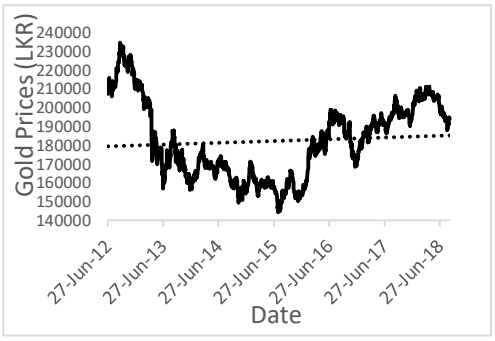


**Figure 3.** Fluctuations in Gold Prices

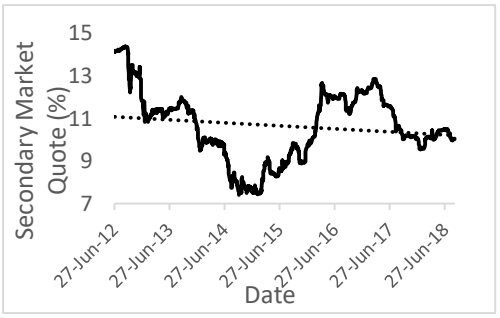


**Figure 4**. Fluctuations in Secondary Market quotes of Treasury Bonds with six years maturity

## Materials and Methods

A portfolio investment is a precise investment process that involves buying and selling of securities. In an investment time horizon, investing in a wide range of asset classes is basically depending on the fact that how the investor would tolerate the risk. Hedging is a strategy that is used to manage the risk. It does not guarantee that the occurrence of adverse outcome will not happen, but having a properly hedged portfolio would result in reduced negative impact. Hedging is defined as risk trading carried out in financial markets, [1] .

In our study, we consider the fluctuations in the stock market and stock data consists of S&P SL20 index prices illustrating performance of the largest and most liquid companies listed at Colombo Stock Exchange. Due to the fluctuations in the stock market, a portfolio investment can be exercised with gold prices, exchange rates and treasury bonds. We consider the gold prices derived at the beginning of the business day at Reuters or Bloomberg and the gold price is based on the price for a Troy Ounce in US Dollars, assuming the standards given by Central Bank of Sri Lanka, 2019.

US Dollar SPOT Exchange rate is a weighted average rate of all actual SPOT transactions executed in the domestic inter-bank foreign exchange market in the previous business day. In our study, US Dollar SPOT Exchange rate is considered as a hedging asset. Treasury bond is a medium and long term debt instrument. In Sri Lanka, as an agent of the government, the Public Debt Department of Central bank of Sri Lanka issues the treasury bonds. As a hedging asset, we consider the secondary market quotes of treasury bonds with six year maturity. Each data set covers a period of 27th June, 2012 to 31st August, 2018. For each data series, continuously compounded daily returns were calculated as $100 * \ln(P_t/P_{t-1})$ where $P_t$ is the daily closing price, [2].

Use of gold prices, exchange rates and treasury bonds for hedging purpose would be beneficial only if they can reduce adverse impacts created by stock market fluctuations.

**Table 1.** Spearman correlations between returns of S&P SL20 index, Gold prices, Exchange Rates and Treasury Bonds

| | S&P SL20 | Gold | Exchange | Treasury Bonds |
|---|---|---|---|---|
| S&P SL20 | 1 | -0.0864 | -0.0266 | -0.0386 |
| Gold | -0.0864 | 1 | 0.1187 | 0.0568 |
| Exchange | -0.0266 | 0.1187 | 1 | 0.0403 |
| Treasury Bonds | -0.0386 | 0.0568 | 0.0403 | 1 |

When considering the Spearman correlations between S&P SL20 index with gold prices, exchange rates and treasury bonds (with six years maturity), they are weakly negatively correlated (Table 1). Thus, it assures that the gold prices, exchange rates and treasury bonds (with six years maturity) can be used as the hedging assets.

*Model*

The return generated by an investment portfolio consisting a spot and futures position can be represented as:

$$R_{H,t} = R_{S,t} - \gamma R_{F,t} \quad (1)$$

Where, $R_{H,t}$ is the return on the hedged portfolio, $R_{S,t}$ are the returns on the spot position, $R_{F,t}$ are the returns on the futures position and γ is the hedge ratio. The variance of the hedged portfolio that is conditional on the information set $I_{t-1}$, at time t-1 is given by;

$$var\left(R_{H,t}\ I_{t-1}\right) = var\left(R_{S,t}I_{t-1}\right) - 2\,\gamma_t\ cov\left(R_{F,t}, R_{S,t}I_{t-1}\right) + \gamma_t^2\ var\left(R_{F,t}I_{t-1}\right) \quad (2)$$

We can obtain the optimal hedge ratios ($\gamma_t$) which minimize the conditional variance of the hedged portfolio. The optimal hedge ratio conditional on the information set $I_{t-1}$ can be obtained by taking the partial derivatives of the variance with respect to $\gamma_t$ and setting the expression equal to zero. [3]

$$\gamma_t *\ I_{t-1} = \frac{cov\left(R_{S,t}, R_{F,t}\middle|I_{t-1}\right)}{var\left(R_{F,t}\ I_{t-1}\right)} \quad (3)$$

The conditional volatility estimates from GARCH models can be used to construct hedge ratios [4]

$$\gamma_t * |\ I_{t-1} = \frac{h_{SF,t}}{h_{F,t}} \quad (4)$$

Where, $h_{SF,t}$ is the conditional covariance between spot and futures returns and $h_{F,t}$ is the conditional variance of futures returns, [2].

*Method*

For each data series, continuously compounded daily returns were calculated as $100 * \ln(P_t/P_{t-1})$ where, $P_t$ is the daily closing price. Based on the above equations, to calculate the hedge ratios, model building was done by estimating Dynamic Conditional Correlation models. This was done by changing the DCC order of the variance equation while considering multivariate T distribution and multivariate normal distribution for the standardized residual distribution. When in building the model, both Dynamic Conditional Correlation (DCC) and Asymmetric Dynamic Conditional Correlation (ADCC) GARCH models were considered.

The selected model based on the Akaike, Bayes, Shibata and Hannan- Quinn criterion was used to calculate hedge ratios. This was done by using the 'rmgarch' package built in R. The method one was to forecast a set of hedge ratios for 30 days' time period by forecasting the calculated hedge ratios using the training set of data. By using the calculated hedge ratios, univariate GARCH models were fitted to each hedge ratio series to simultaneously forecast hedge ratios for a forthcoming 30 days period.

The method two was to forecast a set of hedge ratios for 30 days' time period by forecasting the asset value series for a training set. Then by fitting ARIMA to each individual series after making the series stationary, asset values were forecasted for a forthcoming 30 days period. This forecasted data with the

training set were used to calculate hedge ratio with a similar procedure of calculating hedge ratios described in previous phase.

**Results and Discussion**

In this study, Dynamic Conditional Correlation model of Engle (2002), [5] ,is used to model the volatility dynamics, conditional correlations and hedge ratios between S&P SL20 index , gold prices, exchange rates and treasury bonds. Model building was done by estimating several versions of DCC models. In each specification, a constant term was included in the mean equation and variance equation consisted of GARCH (1, 1). By changing the inclusion of an AR (1) term in the mean equation and changing the distribution as Multivariate Normal distribution (MVN) or Multivariate t distribution (MVT), adjustments were made where the DCC with ARMA (1, 0) term in the mean equation estimated with a multivariate t distribution (MVT) was selected as best fit (Table 2).

**Table 2.** Specifications of the Dynamic conditional correlation model

| | DCC MVN with ARMA(1,0) | DCC MVT with ARMA(1,0) | DCC MVN with ARMA(1,1) | DCC MVT with ARMA(1,1) |
|---|---|---|---|---|
| Akaike | 9.610 | 6.241 | 9.555 | 6.430 |
| Bayes | 9.778 | 6.412 | 9.744 | 6.623 |
| Shibata | 9.608 | 6.239 | 9.553 | 6.428 |
| Hannan-Quinn | 9.673 | 6.305 | 9.626 | 6.502 |

Comparison was done with a graphical view in order to identify the differences created by the two methods compared to the actuals.

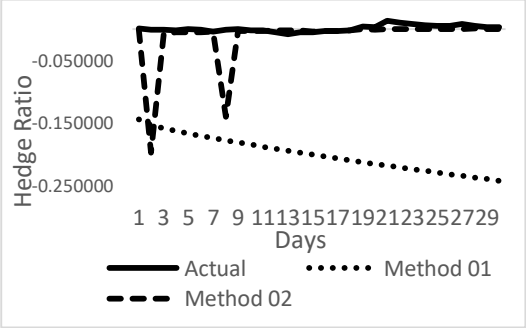


**Figure 5:** Hedge ratios - S&P SL20 index and Gold prices

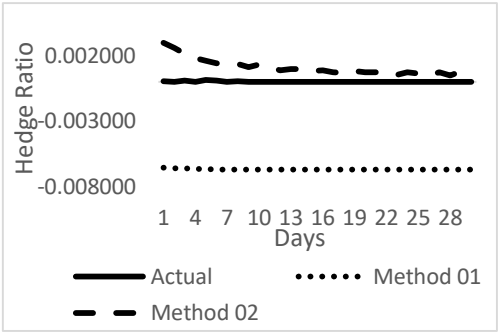


**Figure 6.** Hedge ratios- S&P SL20 and Exchange rates

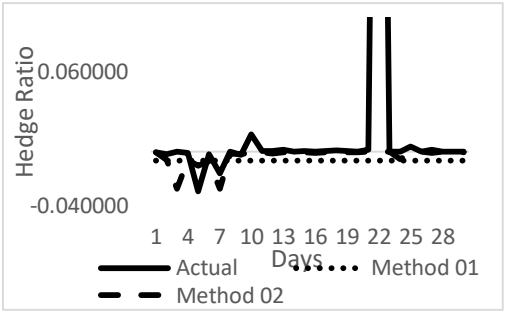


**Figure 7.** Hedge ratios - S&P SL20 and treasury bonds

According to figure 05, 06 and 07, it is evident how the forecasted hedge ratios by using the two methods varying significantly compared to the hedge ratios obtained by the actual data. When comparing the graphical illustration of forecasted hedge ratios by using the two methods, it is evident that the forecasted hedge ratios by forecasting asset values prior to the hedge ratio calculations, which is the method two, seem to be effective compared to the forecasted hedge ratios using method one, in most of the situations.

**Conclusion and Recommendations**

In this study, we found that the S & P SL20 index is negatively correlated with gold prices, exchange rates and treasury bonds (with six years maturity). Thus it assures that the gold prices, exchange rates and treasury bonds (with six years maturity) can be used as the hedging assets, in this context.

The forecasted hedge ratios using the two methods are significantly deviated from the hedge ratios calculated by the actual data. But when comparing the forecasted hedge ratios obtained in both methods, the set of ratios obtained by forecasting the asset values (then by calculating for hedge ratios) instead of forecasting the calculated hedge ratios, seems to be effective to hedge the stock market fluctuations under the considered assumptions. This can be because, hedge ratios may not be a standalone object since they are affected by continuous fluctuations encountered in calculating the hedge ratios. And also, we may say that the hedge ratios cannot be hedged alone due to their correlations since slight changes in stock prices could cause drastic changes in hedging ratios. By the obtained results we suggest that the stock market based on the index S&P SL20, can be hedged by forecasting the hedge ratios by using the forecasted asset values.

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# FORMULATION OF MICRO ENCAPSULATED RUTIN AND EVALUATION OF BIOACTIVITY AND STABILITY UPON IN VITRO DIGESTIVE AND DIALYSIS CONDITIONS

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## Introduction

Rutin is a flavanol glycoside which has received considerable attention as a potential protector against a variety of human diseases such as varicose veins and hemorrhoids. It is also known as vitamin P with excellent antioxidant, anti-inflammatory, anti-diabetic, antiallergenic, anti-viral, and anti-carcinogenic properties and has been demonstrated to scavenge superoxide radicals. However, there are some problems associated with rutin such as low stability, solubility, and low digestion and absorption by intestine and undesirable sensorial features, associated with the direct addition of polyphenols into foods or beverages. The aim of this study is to investigate the effect of encapsulation of rutin with three types of wall materials using three different techniques and their antioxidant activity and bioactivity retention under in vitro gastro intestinal and dialysis conditions, over pure rutin hydrate alone. In this encapsulation process core material is protected from adverse environmental conditions, such as undesirable effects of light, moisture, and oxygen, thereby contributing to an increase in the shelf life of the product and promoting a controlled liberation of the encapsulate.

## Materials and methods

All the chemicals used were obtained from Sigma-Aldrich, St. Louis, MO, USA through Analytical Instrument Pvt Ltd, Colombo, Sri Lanka.

### *Encapsulation of rutin*

Three encapsulation methods were studied such as using starch, egg albumen and lipid carriers.

### *Simulated gastric digestion*

Gastric digestion and Intestinal digestion with dialysis were simulated according to the standard procedure [1].

### *Encapsulation efficiency*

Encapsulation efficiency (EE) was determined using the centrifugation method [2].

*Stability of encapsulated rutin by HPLC*

The concentration of rutin in encapsulated rutin and pure hydrate were determined using HPLC (LC- 20AT, SHIMADZU, Japan) for undigested, digested, after intestinal phase and dialyzed samples. This was done injecting 20µl of filtered samples which were diluted with equal amount of mobile phase (same mobile phase mentioned above).

*Bioassay*

*ABTS radical scavenging assay* was carried out according to the protocol described in a previous method [3]. Singlet oxygen scavenging assay was carried out according to the protocol described by [4], with some modifications [3]. Lipoxygenase Inhibition Assay was carried out according to the protocol described by [5] with some modifications.

*Particle size and polydispersity index analysis*

The average particle size and polydispersity index of Rutin encapsulated in lipid carrier was measured by the particle size analyzer (Zetasizer Ver. 7.03 from Malvern Instruments Ltd at SLINTECH, Sri Lanka) at room temperature.

*Structure elucidation of Rutin encapsulated in lipid carrier by fourier transform Infrared (FTIR) spectroscopy*

Infrared spectra of the molecules Rutin encapsulated in lipid carrier was measured using Fourier Transform Infrared (FTIR) spectrums of prepared samples using Shimadzu IRAffinity-1S, Japan (Department of Nano Technology, Wayamba University of Sri Lanka) in its native form as an emulsion.

*Statistical analysis*

All the three encapsulated rutin samples and pure rutin hydrate were analysed and resulted values were presented as mean±SD (Standard Deviation). Statistical calculations were carried out by SAS System. One-way ANOVA was applied to determine the statistic differences. Mean separation was also carried out by LSD MEANS Procedure. $P<0.05$ was considered as significantly different.

**Results and discussion**

The encapsulation efficiency of Rutin encapsulated in lipid carrier was significantly higher than that of the Rutin- starch complex and Rutin encapsulated in egg albumin. The difference in the encapsulation efficiency could be due to the differences in wall material and extent of interactions occurring between wall material and Rutin molecules, which could facilitate the incorporation of rutin in lipid globules.

From bioassays, results showed that the rutin encapsulated lipid carrier has the highest ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical inhibition activity for all the digestive phases; (1.697± 0.032 mg RE/mL for

digested fraction after gastric phase, 1.767±0.027 mg RE/mL after intestinal phase and 0.623± 0.012 mg RE/mL after dialysis); compared to all other samples. It also has the highest singlet oxygen scavenging activity after gastric phase compared to all other samples (0.383±0.003 mg RE/mL). It has the highest anti-inflammatory activity for dialyzable fraction which was lowest in undigested sample (undigested sample 8.033 ±0.033% and dialyzable fraction 17.80±0.153%).

HPLC results revealed that rutin content of encapsulated lipid carrier for all the digestive phases were significantly higher (P≤0.05) than that of all other encapsulated samples. Results indicate that encapsulation on rutin can improve the availability of rutin after digestive conditions which is beneficial for the targeted delivery of rutin as a drug.

*Particle size and polydispersity index analysis*

The rutin encapsulated lipid carrier has average droplet size at 1704 ± 1015.953 nm with a polydispersity index (PDI) of 0.909 showing scattered size distribution when diluted with hexane. It also had average droplet size at 2474 ± 740.905 nm with a polydispersity index (PDI) of 1.000 showing even more scattered size distribution when diluted with its oil base. it is known that increasing oil concentration increases particle size which opposes clear emulsion formation [4].

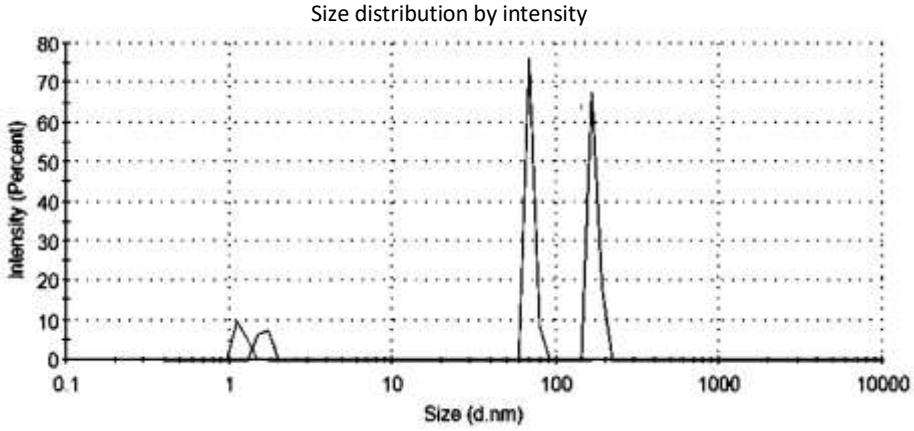


**Figure 1**. Droplet size and size distribution of rutin encapsulated lipid carrier when diluted with hexane

*Structure elucidation of Rutin encapsulated in lipid carrier by fourier transform Infrared (FTIR) spectroscopy*

The possible interaction between rutin and lipid phase in the Rutin encapsulated in lipid carrier is indicated by FT-IR spectroscopy and presented in Figure 02. These positional as well as morphological changes in the peaks can prove the physical or ionic interaction occurring between rutin and lipid carrier and complete incorporation of the drug within lipid carrier.

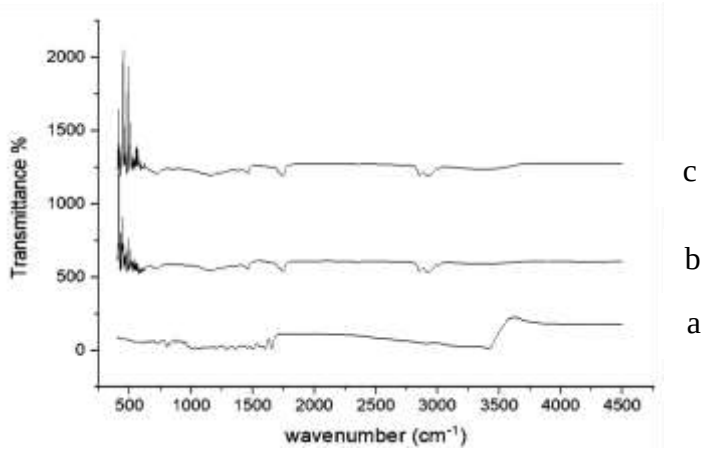


**Figure 2**. FTIR spectra of the rutin (a), rutin encapsulated lipid carrier (b) and lipid carrier alone (c)

## Conclusions and Recommendations

Lipid carrier has significantly increased the ABTS radical scavenging ability, Singlet oxygen scavenging ability and Lipoxygenase Inhibition percentage of rutin which indicates the lipid carrier can be a possible effective carrier material for the oral delivery of rutin. The information contained in the FTIR spectra of rutin encapsulated lipid carrier revealed the possible interaction between rutin and lipid carrier which ensures successful encapsulation. Scanning electron micrographs shows that rutin encapsulated lipid carriers present at microscale thus ensures the microencapsulation. Developed micro encapsulants of rutin could provide a method for designing new functional foods based on microcarriers.

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# CHARACTERIZATION OF MANUFACTURED AGGREGATED POROUS MEDIA AS PLANT GROWTH SUBSTRATES

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## Introduction

Manufactured Porous media have gained increased interest as plant growth substrates in current-day agricultural and horticultural applications due to their pronounced growth supportive properties. Manufactured porous media is of great diversity depending on their raw material, aggregate size, density, structural arrangement, and pore network configurations [1]. Despite wide variability, most of the aggregated porous media exhibits two distinct regions of pores, i.e., Inter-aggregate pores-porosity among aggregates and Intra-aggregate pores – pores within individual aggregates where available total pore space is mutually shared by air and water [1]. The presence of three distinct phases essentially induces major control over plant growth requirements such as aeration, water availability, nutrient transport and temperature variation. In a given porous medium, soil aeration is typically expressed in terms of gas diffusivity ($D_p/D_o$ where $D_p$ ($cm^2$ $s^{-1}$) and $D_o$($cm^2s^{-1}$) are the gas diffusion coefficients in considered media and free air under identical pressure temperature conditions, respectively), water availability is explained by media water retention characteristics, nutrient (solutes) transport is expressed as solute diffusivity or its proxy, for example relative permittivity ($E/E_o$ where E is the permittivity under unsaturated condition and $E_o$ is the saturated permitivity), and the temperature variation by means of Thermal conductivity ($\lambda$, W $m^{-1}$ $K^{-1}$).

Accurate experimental determination of media properties requires specific apparatus and boundary conditions which are expensive for frequent repetitions. Therefore, the use of predictive models estimating each parameter from easy-to-measure media properties (air-filled porosity (Ɛ, $cm^3cm^{-3}$) and total porosity (Ø, $cm^3cm^{-3}$)) is commonly practiced. Although the literature is about with studies characterizing properties individually, a comprehensive study applying bimodal numerical models integrating all four properties for characterization of suitable growth media are rare [2]. In this study, two manufactured aggregated media were characterized for SWC, gas diffusivity, thermal conductivity, and permittivity using recently and newly developed

bimodal models for obtaining a better insight in behavioral patterns of aggregated growth media.

## Materials and Methods

Two manufactured growth media including NASA-developed Zeophonic (0.25-1.0 mm) and Turface (2.0-5.0 mm) were selected from literature representing a wider range of total porosity with the media properties as shown in Table 1 below.

**Table 1.** Growth media properties and data reference

| Growth Media | Φ ($cm^3$ $cm^{-3}$) | $\rho_b$ (g $cm^{-3}$) | Raw material | Reference |
|---|---|---|---|---|
| Zeophonic (0.25-1.0 mm) | 0.61 | 0.97 | Zeolite-based | This study/ [2] |
| Turface (2.0-5.0 mm) | 0.75 | 0.62 | Baked ceramic | |
| Φ – Total porosity | | | $\rho_b$ – Bulk density | |

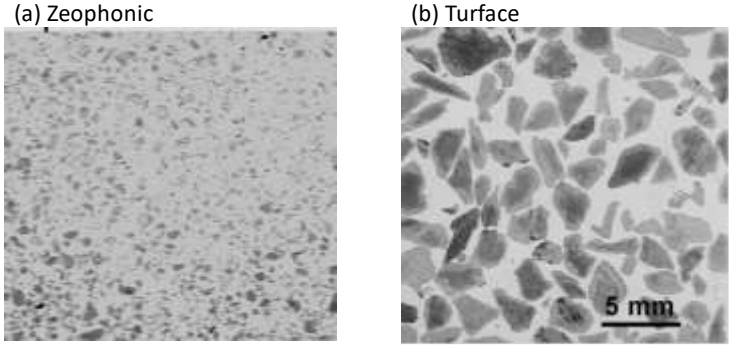


**Figure 1.** SEM images of (a) Zeophonic and (b) Turface. (Image curtsey – [2])

For determination of gas diffusivity ($D_p/D_o$), two growth media were repacked in to 100 $cm^3$ annular cores to the desired bulk densities (Table 1) and saturated under water for 72hrs. $D_p/D_o$ measurements and calculations followed the method introduced by Taylor 1949 using one-chamber diffusion apparatus [3] at different moisture contents achieved by stepwise evaporation of saturated samples. Soil water retention was obtained by sequential draining inside sand boxes (pF <2), pressure plate apparatus for (pF >2) and WP4C dew point potentiometer for dry end measurements. Dielectric permittivity was measured using time domain reflectrometry (TDR) measurements by means of a custom-designed measurement cell with a parallel-plate probe arrangement where samples were first vacuum saturated with NaCl solution and packed in to the cell for measurements [2]. Thermal conductivity was measured using a small cell apparatus equipped with soil moisture ($ECH_2O$ EC-5, Decagon Devices) thermal conductivity (SH-1, Decagon Devices) and capillary pressure (porous cup tensionmeter) sensors which wet-packed samples were drained to desired matric potential levels and measurements were taken [4].

*Numerical Modelling*

For Numerical simulation of each of the properties considered, recently developed models were used as described in Table 2.

**Table 2.** Growth media properties and data reference

| Numerical Model | Reference |
|---|---|
| Water Retention Characteristic – Bimodal van Genuchten water retention function<br>$$S_e = \frac{\theta - \theta_r}{\theta_s - \theta_r} = \sum_{i=1}^{2} w_i \left(\frac{1}{1 + |a_i \psi|^{n_i}}\right)^{m_i}$$<br>Pore size distribution – First derivative of water retention function<br>$$\frac{d\theta(r)}{dlog_{10}r} = \frac{d\theta(pF)}{d(pF)} = \frac{d\theta(\psi)}{dlog_{10}|\psi|} = \frac{d\psi}{dlog_{10}|\psi|} = \frac{d\theta(\psi)}{d\psi} = [log_e 10]\psi\left|\frac{\partial\theta}{\partial\psi}\right|$$ | [5] |
| Gas Diffusivity<br>For Region 1: $\frac{D_p}{D_o} = \frac{\alpha_1}{w^{\beta_1}}\left(\frac{\varepsilon}{\emptyset}\right)^{\beta_1} \quad \varepsilon \leq w\emptyset$<br>For Region 2: $\frac{D_p}{D_o} = \alpha_1 + \frac{\alpha_2}{(1-w)^{\beta_1}}\left(\frac{\varepsilon - w\emptyset}{\emptyset}\right)^{\beta_2} \quad \varepsilon \leq (1-w)\emptyset$ | [1] |
| Thermal conductivity – Modified Cote and Konrad model<br>For Region 1: $\lambda(K_{e,i}) = (\lambda_{sat} - \lambda_f)K_{e,i} + \lambda_f \quad S_o \leq S \leq S_f$<br>$$K_{e,1} = \frac{S - S_o}{S_f - S_o}$$<br>For Region 2: $\lambda(K_{e,i}) = (\lambda_f - \lambda_{dry})K_{e,i} + \lambda_{dry} \quad S_f \leq S \leq 1$<br>$$K_{e,2}(S) = \frac{k(S - S_f)}{1 - [1 - k(1 - S_f)]S}$$ | [4] |
| Relative Permitivity<br>For Region 1: $\frac{E}{E_o} = A(\theta)^B \quad \varepsilon \leq w_2\emptyset$<br>For Region 2: $\frac{E}{E_o} = A(w_2\emptyset)^B + C(\theta - w_2\emptyset)^D \quad \varepsilon \leq (1-w_2)\emptyset$ | This study |

| | |
|---|---|
| θ- moisture content ($cm^3cm^{-3}$) | r- pore radius (cm) |
| $\theta_s$-saturated moisture content ($cm^3cm^{-3}$) | Ψ – matric potential, pF=log(-ψ, cm $H_2O$) |
| $\theta_r$-residual moisture content ($cm^3cm^{-3}$) | λ , $\lambda_f$, $\lambda_{dry}$, $\lambda_{dry}$ – thermal conductivity, thermal conductivity related to $S_f$, dry thermal conductivity, saturated thermal conductivity ($Wm^{-1}K^{-1}$) |
| Ɛ- air-filled porosity ($cm^3cm^{-3}$) | |
| Ø-total porosity ($cm^3$ $cm^{-3}$) | |
| $D_p$, $D_o$ – gas diffusion coefficients in porous media and free air ($cm^2$ $s^{-1}$) | $K_{e,i}$(i=1,2) Kersten number for region 1, 2 |
| $n_1$, $n_2$, $m_1$ (=1-1/$n_1$), $m_2$ (=1-1/$n_2$), $\beta_1$, $\beta_2$ – shape factors | S=θ/Ø, $S_f$, $S_o$ – saturation, considered saturation, zero saturation |
| w=$w_1$, $w_2$ are weighting factors for region 1 &2 | E – permittivity in unsaturated media |
| $a_1$, $a_2$, $\alpha_1$,$\alpha_2$ – model scalling factors ($cm^{-1}$) | $E_o$ – Permitivity in Saturated condition |
| | A,B,C,D – Model fitting parameters |

## Results and Discussion

*Water Retention Characteristics (WRC) and Pore Size Distribution (POSD)*

Shown in Figure 2 are (a) the water retention and (b) the pore size distribution for the two growth media. As adequately described by the bimodal type van Genutchen water retention function, both media showed two air-entry values in their WRC curves indicating strong dual-porosity nature. Turface (2.0 – 5.0 mm) being large in aggregate size than Zeophinic (0.25 – 1.0 mm), initially held more water in its large interaggregate pore network while increase in matric suction (~10 cm $H_2O$) suddenly drained pore water entering air in to the media interaggregate pores. Comparatively, smaller pores in Zeophonic held lesser amount of water under low matric suctions but amount of water held at dry end is higher than that for Turface in its intraaggregates pores which are smaller than pores of Turface. Importantly, the boundary demarcating between two pore regions for Zeophonic ($\theta$~0.22 $cm^3cm^{-3}$), and Turface ($\theta$~ 0.33 $cm^3cm^{-3}$) are denoted by second air entry values.

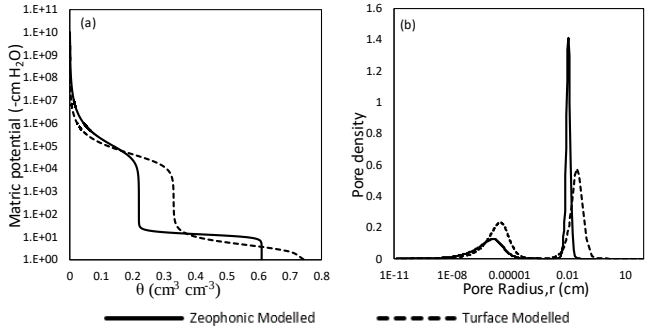


**Figure 2.** (a) Water Retension Characteristics and (b) Pore Size Distribution for Zeophonic (soilid line) and Turface (dashed line)

Bimodality of the two growth media are further exhibited in the POSD in Figure 2(b) with two density peaks in each curve respective to two distinct pore regions, i.e., peak with large diameter (r) correspond to interaggregate pores and smaller diameter correspond to intraaggregate pores. As the curves evidenced, the inter aggregate pore size of Zeophonic is smaller than Turface resulting lesser space for water to be held under low matric suctions while smaller intraagretate size in Zeophonic is advantageous for plants in holding water under higher suction levels.

*Gas Diffusivity ($D_p/D_o$), Solute Diffusivity ($E/E_o$) and Thermal conductivity ($\lambda$)*

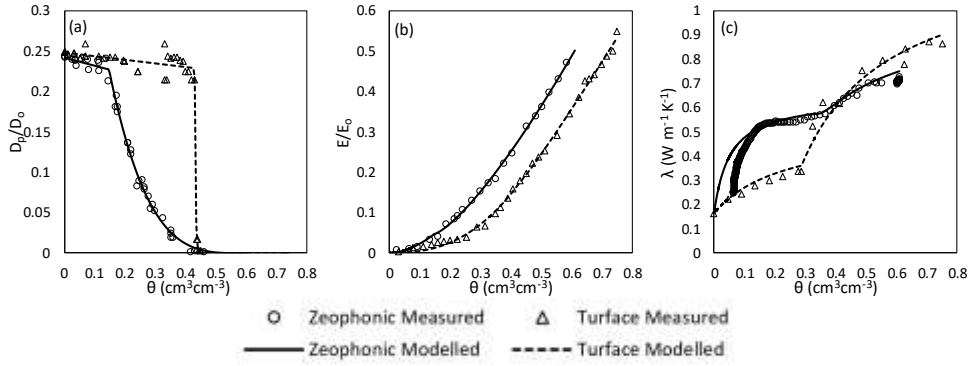


**Figure 3**. (a) Gas diffusivity ($D_p/D_o$) (b) Solute diffusivity ($E/E_o$) and (c) Thermal conductivity ($\lambda$) for Zeophonic (circles) and Turface (traingles) with fitted curves (soild and dashed lines respectively)

The parameterized Gas Diffusivity, Solute diffusivity and Thermal conductivity of the two media are shown in Figure 3 as a function of moisture content ($\theta$). Gas diffusivity which increases with decrease in moisture content, showed a clear bimodal behavior as well parameterized by suggested Two-region model (Figure 3 (a)). Under low moisture conditions, both the media showed higher diffusivities, progressive wetting kept decreasing diffusivity slightly up to complete saturation of intraaggregate pores. Sudden drop in diffusivities were as a result of moisture retention and resulting bridging in inter-aggregate water held [1].

Solute Diffusivity ($E/E_o$) for both media showed bimodal behavior albeit to a lesser extent. Note that $E/E_o$ increases with increase in hydraulic connectivity through pore structure [2]. The increase with increase in $\theta$ was initially slow with moisture available in interaggregate pores, while a rapid increase was observed with water intrusion in to intraaggregate pores.

The thermal conductivity ($\lambda$), on the other hand, is driven by grain-grain connectivity and therefore $\lambda$ increased with the decreasing porosity [4]. Conductivity under fully dry condition ($\theta$=0) is a result of solid aggregate connections which progressively increased when the pore air (with less conductivity) is replaced by more conductive water.

It is worth to notice that property variations are quite analogus to $\theta$ variations controlled by pore structure where all the graphical deviations occurred near the second air entry values with minor shifts arise due to numerical simulations.

**Conclusions and Recommendations**

This study characterized hydraulic, aeration, solute and heat transfer properties of two manufactured aggregated porous media to be used as prospective plant growth substrates. Both media showed structural bimodality with two distinct pores regions with respect to all measured properties, which were adequately described by proposed parametric functions. The study thus gives a valuable implication on structural suitability of the two media as growth substrates.

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# EFFECT OF ANODIZATION VOLTAGE AND TIME ON THE STRUCTURE OF $TiO_2$ NANOTUBE ARRAYS

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## Introduction

In materials science, $TiO_2$ nanotube arrays have taken more attention than other $TiO_2$ Nanostructures due to its unique properties such as higher charge transport capability by their vertically aligned porous nanostructures and excellent chemical and mechanical properties. They are used in advanced functional devices such as solar cells, supercapacitors, gas sensors and in biomedical applications for better performance [1]. Functional properties required in these applications of $TiO_2$ nanotube arrays are unique to the application and they can be easily manipulated by changing the nanotube morphology [2].

The most promising synthesizing process for highly ordered $TiO_2$ nanotubes is the electrochemical anodization process. Resulting tube morphology of $TiO_2$ nanotubes formed by anodization method is highly sensitive to the process parameters. But the published data shows a wide discrepancy in this relation between process parameters and tube morphology. Therefore, it is important that the synthesizing process of $TiO_2$ nanotubes is tailor-made to suit the specific requirement. The most affecting two process parameters are identified during the literature review. That are, anodization voltage and anodization time [1],[3]. The research will optimize the synthesis process of $TiO_2$ nanotubes for in-house preparations based on those two key process parameters.

## Materials and Methods

10 mm × 20 mm size Ti foil (0.5 mm thickness) was polished with sandpaper and degreased by sonication in ethanol, DI water respectively. Ethylene Glycol electrolyte is prepared to have 0.3% wt $NH_4F$, 0.5% DI water. Anodization is carried out using the Pt electrode as cathode and Ti foil as the anode with a 30 mm electrode distance. Voltage and time parameters are varied as stated in Table 1 for different experiments to analyze the tube morphology variations accordingly. The temperature of the electrolyte was kept at 25 $^0$C by using an ice bath. After taking out from the anodization cycle the sample was soaked in Ethanol for 15 minutes. Samples are annealed at 450 $^0$C with a 1 $^0$C per minute temperature increment rate and furnace cooling (temperature increased to 450 $^0$C within 3 hour and 45 min and the soaking time was 1 hour).

The as-prepared samples were characterized using the scanning electron microscope. The diameters of the nanotubes were measured using the scanning electron microscopic images obtained.

**Results and Discussion**

*$TiO_2$ formation on the Ti foil*

Figure 1 shows the XRD spectrum of an annealed sample. The characteristic peaks of the Anatase and Rutile phases observed confirmed the formation of $TiO_2$ on the Ti foil during anodization. Around $58^0$-$65^0$ there can be seen a broad peak. Since the as-prepared $TiO_2$ is amorphous this peak can be a result of remaining amorphous after $TiO_2$ annealing.

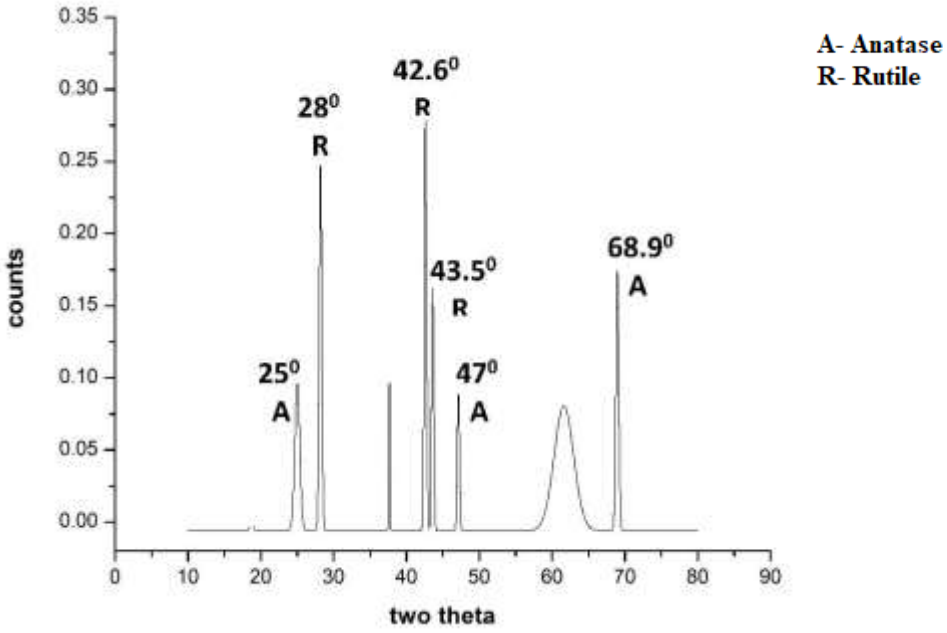


**Figure 1.** XRD spectrum of an anodized sample after annealing

*Morphology of the $TiO_2$ nanotube layer*

Samples with 1 hour and 2 hour anodization times show a tube mesh-like morphological structure on the top (Figure 2). But separated tubes are visible in all 3 hour samples. Samples of 3 hour anodization time conclude a general trend that the longer time periods will result in separated tube structures than a tube mesh. The reason for this phenomenon is the new voids starting to form between the pores with longer time periods [4].

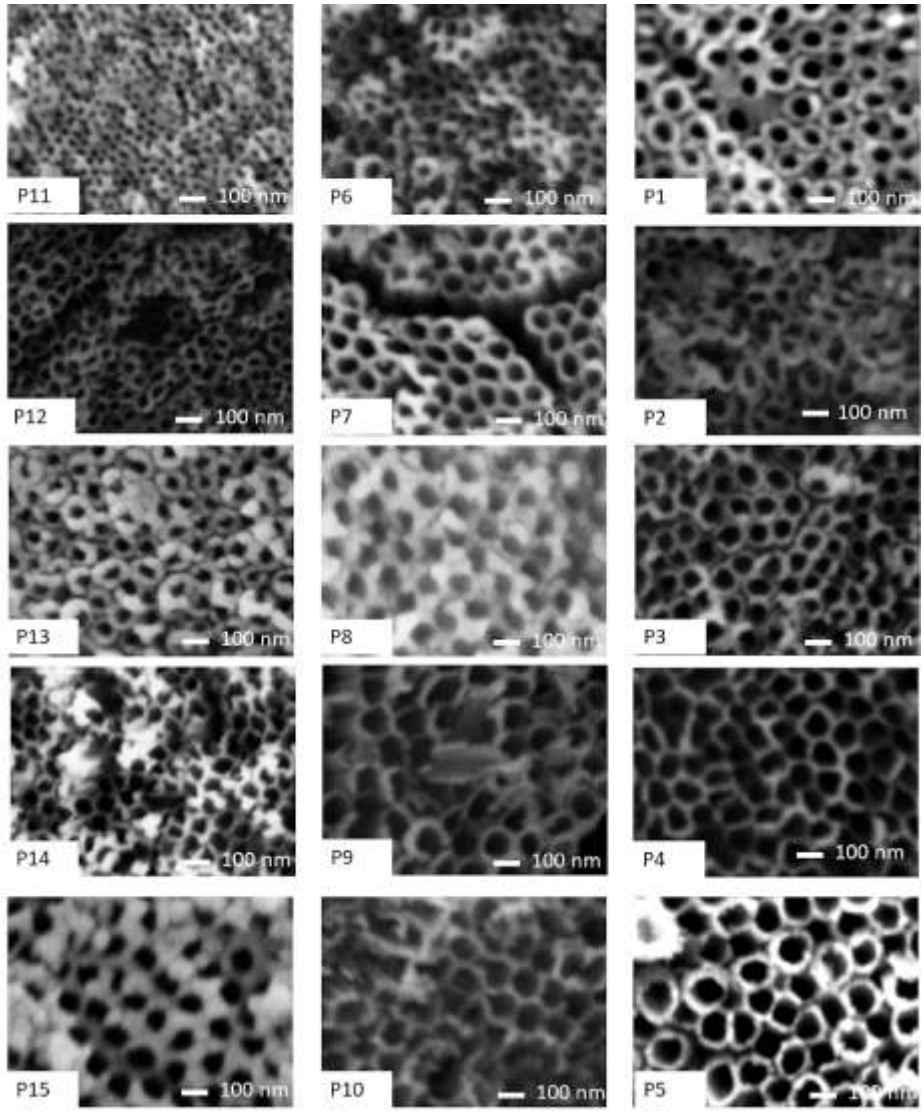


**Figure 2.** SEM images of the top structures of the 15 samples

**Table 1.** Process conditions and tube morphology data

| Sample | Voltage (V) | Time (hours) | Tube diameter(nm) | |
|---|---|---|---|---|
| | | | Average | SD |
| P1 | 20 | 3 | 36.03 | 6.85 |
| P2 | 30 | 3 | 52.48 | 9.78 |
| P3 | 40 | 3 | 66.02 | 13.47 |
| P4 | 50 | 3 | 73.78 | 8.00 |
| P5 | 60 | 3 | 89.38 | 12.24 |
| P6 | 20 | 2 | 32.19 | 6.24 |
| P7 | 30 | 2 | 56.51 | 8.57 |
| P8 | 40 | 2 | 64.30 | 9.54 |
| P9 | 50 | 2 | 74.41 | 8.69 |
| P10 | 60 | 2 | 81.63 | 15.62 |
| P11 | 20 | 1 | 28.58 | 6.38 |
| P12 | 30 | 1 | 44.03 | 5.89 |
| P13 | 40 | 1 | 50.38 | 8.14 |
| P14 | 50 | 1 | 62.21 | 11.56 |
| P15 | 60 | 1 | 63.87 | 7.71 |

According to Figure 3, a linear trend can be seen in all 5 voltage stages. Also, the diameters are in different nanometer ranges for the five different voltage values. This concludes that there is an effect from the voltage on the diameters such that the diameters increase with voltage. According to the growth mechanism of nanotubes, the initial pits formed by etching of the initial compact $TiO_2$ layer are determining the pore diameters. If the voltage is high, then the electric field associated is high which means higher etching rate at the initial stage. This causes higher diameter pit formation which later grows into nanopores [5]. The effect of the etching rate on tube diameter is lower after this initial pit formation incident. Therefore, the effect of time on pore diameter is lower compared to voltage. Also, it can be observed that the 2 hour and 3 hour diameter values are in an overlapping region which shows signs of diameter saturation with increasing.

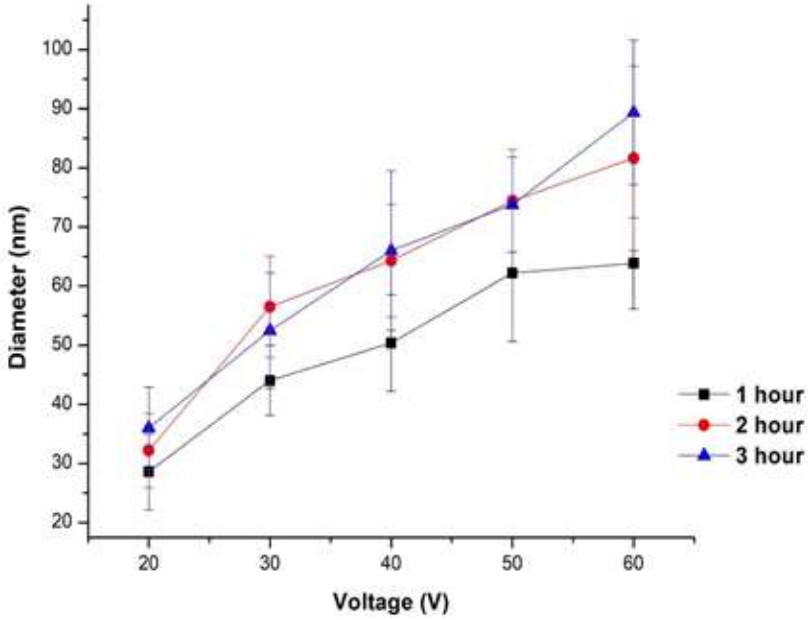


**Figure 3.** Voltage vs Tube diameter graph

## Conclusions and Recommendations

The synthesized nanotubes are in the range of 28 nm to 90 nm. There is a strong relationship of tube morphology to voltage and time which is the increment of diameter with both voltage and time. Separated tubes can be obtained with longer anodization time periods. Also, the increasing of diameter is reaching a saturation level with time. The expansion of the study to higher time periods more than 3 hour will be needed to confirm this behavior. Since there is a strong relationship, the tube morphology can be tailor made to the functional properties required in the application where the $TiO_2$ Nanotube layer is used by changing the synthesis process parameters.

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# DEVELOPMENT OF A RELIABLE METHOD TO QUANTIFY THE SOLDERABILITY OF LEAD-FREE SOLDER ON NICHROME ALLOY

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## Introduction

Nichrome (Ni/Cr) alloys are used in many technological applications for instance, electrical resistors, heating elements and electrical sensors, and in resistive strain gage manufacturing. The main motive behind using nichrome alloy for such application is its favorable material properties[1] such as low temperature coefficient of resistance (85 ppm/k) and large resistivity (110 μΩ.cm). Its commercial availability and low dependence of gage factor makes this an even more attractive choice for electrical resistor-based sensors constructions. It has been shown that the surface energy of nichrome alloy is altered by the formation of a passive layer on the alloy and it inhibits the wettability on nichrome surface.

As there are no direct measurement methods to assess surface energies of a solid interface, indirect methods have been employed to carry out the task. The contact angle measurement is considered to be the simplest among those indirect measurement methods.

The contact angle evaluation was carried out using software developed based on 'Visual Basic' programming language. The principle objective of this study is to introduce an industrial friendly technique to enhance the solderability on nichrome alloy.

## Materials and Methods

*Contact angle measurements*

The wettability of a surface is directly related to the surface energies and surface energies are linked through Young equation.

$$\sigma_s = \sigma_{sl} + \sigma_l \, Cos\theta \quad (1)$$

This equation contains four independent parameters. The contact angle θ and the surface free energy of the liquid ($\sigma_l$) are the only measurable parameters, surface free energy of the solid( $\sigma_s$) and surface energy of solid-liquid interface($\sigma_{sl}$) can only be determined by the so-called adhesive tension or wetting tension ($\sigma_s$ and $\sigma_{sl}$). The equation also shows that, the high solid surface

free energy, low interfacial free energy and low liquid surface free energy make surfaces more wettable.

The measurements of contact angle of solder droplets were done by the developed software. This software allows user to fit a polynomial curve or an ellipse to the droplet phase boundary by constrained nonlinear regression methods.

For the contact angle measurement, the droplet image has to be loaded to the software in any common digital image formats (JPEG, PNG). Then the base line for the droplet has to define by the user. User has to select the fitting method; a) Polynomial fitting or b) ellipse fitting and the programme converts these data into equations. Equations generated by the programme are solved automatically to find the roots, thereby it obtains the accurate contact positions and then calculate the slope at these contact points.

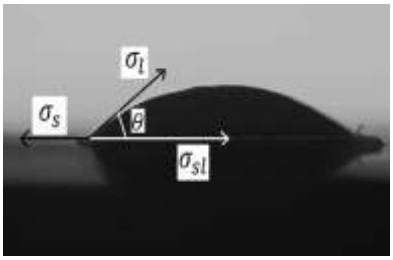


**Figure 1.** Wetting of A Surface: Geometrical Basis of Young's Equation

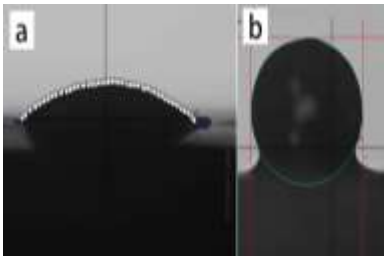


**Figure 2.** Contact Angle Calculation Processed By (a) Polynomial Fitting Method (b) Ellipse Fitting Method

Polynomial root finding algorithm is based on the Jenkings/Traub method. The ellipse fitting is the other fitting method included in the programme. Here user has to define the base line and three distinct points on the curved droplet surface for the program to generate the ellipse. Drop shape fitting procedure by the developed programme is illustrated in Fig.2.

A comparison-based validation process was carried out to examine the accuracy of the software. This process involves two proof bodies with known contact angles. A comparison of contact angle values taken from geometrical relations and developed software shows that this method of image analysis is accurate. This validation process is well defined in our previous work[2].

*Sample preparation*

Ten 1mg of solder materials were placed on a thin nichrome film of 10mm× 13mm x 6µm and heated to 325-350°C using a hot plate. The nichrome film was kept at 325-350°C temperature for about 8 seconds. The temperatures used were well above the melting temperature of solder material (which is about 227°C) to ensure complete melting. The primary goal of this process was to form solder dots on the nichrome thin film. Once the droplets were formed the

solder dots were separated from the nichrome thin films by cutting out approximately 3mm× 3mm pieces. These thin film pieces were then glued to an edge of a flat surface and captured the images of them from a microscope at 50× magnification (see Fig-3(a)). Images taken from this method are shown in Fig-3(b).

Captured images of solder droplets were loaded to the developed software and the contact angles were calculated. The same procedure was followed for a $ZnCl_2$/HCl pre-treated nichrome film.

Contact angles of solder droplets were calculated by changing the HCl content of the etchant. 10 samples sets were tested, and contact angles were measured for each percentage change. Data obtained are summarized in Fig.5.

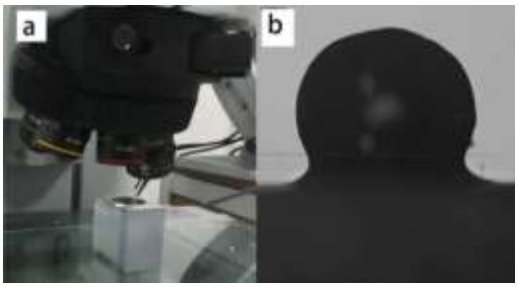


**Figure 3.** (a) Solder dots observation using a microscope, (b) captured image of a solder dot by microscope

**Results and Discussion**

Figure 4 shows the images obtained of solder droplets of treated (By $ZnCl_2$/HCl) and non-treated samples. These images were fed to the program and contact angles were calculated. Data obtained are summarized in Fig-5. The data shows that the values of contact angles of solder droplets were changed from from average of 118.7° to 26.4° after treating with $ZnCl_2$/HCl by changing the HCl content.

There is a passive chromium oxide layer formed on the nichrome surface[3]. Aqueous salts such as $ZnCl_2$/HCl, used in this experiment wet this oxide layer and penetrate through it due to capillary action. This penetration mainly occurs through pores and cracks. The salts dissolve metal ions on the outside surface and facilitate oxidant penetration into the metal. This degradation process produced metallic chlorides and they may form above or below of the metal-oxide interface. These metallic chlorides move into the melt-gas interface as dissolved components. This degradation process is accelerated by acidic environments.

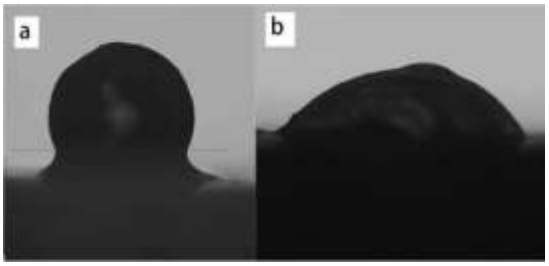


**Figure 4.** Images of solder droplets taken (a) without the $ZnCl_2$/HCl treatment (b) with the $ZnCl_2$/HCl treatment

A similar type of study has been carried out by Porcayo et al[4]. They studied corrosion resistance of nichrome (80Ni/20Cr) at the presence of $ZnCl_2$-KCl molten salts and they have clearly concluded that the formation of $Cr_2O_3$ layers on nichrome (80Ni/20Cr) alloy is protecting the base metal from environments. They also showed that this oxide layer may get damaged internally at high temperatures. The solubility of $Cr_2O_3$ layer is increasing in the presence of $Zn^{2+}$ ions. The $Cl_2$ diffuse into the alloy directly reacts with the alloy itself. These direct reactions are solid-gas reactions.

$$Cr_2O_3 + 3Cl_2 \rightarrow 2CrCl_3 + \frac{3}{2}O_2 \quad (2)$$

$$ZnCl_2 + Cr_2O_3 + \frac{1}{2}O_2 \rightarrow ZnCr_2O_4 + Cl_2 \quad (3)$$

Furthermore, in low oxygen pressures, $Cr_2O_3$ is stable in the NI/Cr system. When the $Cl_2$ partial pressure increases, $Cr_2O_3$ is unstable and can be converted into metallic chlorides.

The results obtained from this study are in agreement with the above explanations. And it is also observed that the contact angle is reducing with the HCl content of the etchant.

These experimental results highlight the fact that the formation of passivation layer on nichrome alloy is strongly contributed to the inhibition of wettability on alloy surface. It was confirmed by this study that the passivation $Cr_2O_3$ layer can also be removed using $ZnCl_2$/HCl, pre-treatment.

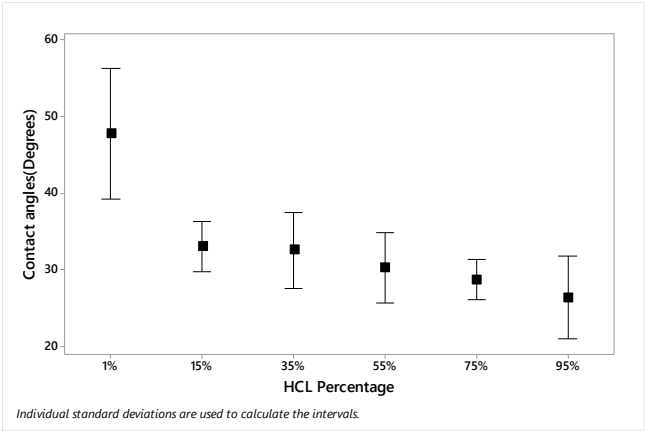


**Figure 5**. Resultant contact angle values of solder droplets after the treatment of $ZnCl_2$/HCl by changing the HCl content.

Comparison test carried out in this study is a commonly used validation method in order to ensure the quality and precision of a measuring system. Our previous works concluded that MAE (Mean absolute error) value of measured contact angles between proposed image processing algorithm and geometrical relations is lower than 1%.

Which makes this proposed method of image analysis is suitable for take precise readings [2]**.** Which make this method superior to calculating contact angles by direct measurements from an image.

According to the results of the present study, wettability of nichrome (Ni/Cr) thin films can be enhanced by, using $ZnCl_2$ in an acidic environment (HCl), by degrading passive oxide layer consisting $Cr_2O_3$. And the wettability can be further enhanced by increasing the HCl content of the etchant.

Therefore, the solderability of nichrome (Ni/Cr) thin films can be enhanced (At 325°C-350°C) by this method.

**Conclusions**

The experimental results confirm that the solderability of the nichrome (Ni/Cr) surface can be enhanced by removing the passivation layer. This passive oxide layer removal can be done by increasing the $Cl_2$ partial pressure on the nichrome surface and increasing the solubility of oxide scale. Molten salts such as $ZnCl_2$ in acidic environment is suitable for this purpose.

The solderability on nichrome (Ni/Cr) substrate can be further enhanced by increasing the HCl content of the etchant used in this study ($ZnCl_2$/HCl).

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# INTEGRATING CUSTOMER REQUIREMENTS WITH PRODUCT DESIGN IN THE DEVELOPMENT OF CUSTOMER ORIENTATION: A CASE STUDY OF GLOVE MANUFACTURING IN SRI LANKA

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## Introduction

Any organization must give priority to the quality of its products while satisfying the needs and wants of the customers. Often it is difficult to identify real customer requirements and meet them. Even though the organization identify the customer requirements, transferring those into the engineering team or the product design teams is difficult with the absence of a proper mechanism for that. This study is focused on one of the major glove manufacturing companies in Sri Lanka, which is currently facing the problem of not having a proper mechanism for transforming customer requirements into product designs. Since the products are exported and the customers are not easy to reach, customer needs and wants are difficult to identify. Hence, the quality of the final product may not be at the expected level. This issue has led to increase the number of customer complaints and to decrease the quality of the products. Moreover customer retention is extremely important to any organization, hence need to satisfy them by providing quality products. In order to achieve customer satisfaction, the needs of customers should be fulfilled. To enable this, the customer should be integrated into the product development process. The objectives of this research were to identify the weaknesses of the existing designing process and recommend strategies to improve product design. For this study methodology, QFDs for three styles of a single product series is prepared and the results are evaluated using statistical tests.

## Materials and Methods

In order to achieve sustainable competitive advantage in the market, it is necessary to provide and improve customer satisfaction [1]. Therefore, the customer has become great importance to companies and many businesses pay attention towards customer satisfaction.

Customer requirements, expectations, and needs of them are important factors of customer satisfaction. When the customer requirements are achieved, customer satisfaction and loyalty are also achieved [3]. Customer requirements arise from needs and these needs of customers are part of their expectations. Understanding and fulfilling the needs of customers have been well recognized

as one of the main factors for product design and development to be succeed [2].

Quality Function Deployment (QFD) is referred to as the most advanced customer satisfaction model [5]. In this approach, customers and the process are focused rather than the product. Quality refers to meeting customer requirements. The primary functions of the QFD are product development, quality management, and customer needs analysis. Later, the functions have been expanded to wider fields and there is no fixed limitation for these fields of applications. Therefore, QFD can be considered as a link between customers, design engineers, competitors, and manufacturing [4].

House of Quality (HOQ) is a popular design tool that is supportive in information processing and in decision making of the engineering design process. For the companies that are just implementing QFD and the HOQ, there is undoubtedly an improvement in information structure, flow, and direction. The HOQ is the structure or graphical summary of QFD. It consists of a set of different matrices.
The study is focused on transforming customer ideas into new product development and increasing the quality of the products using QFD. This is a qualitative research. For this study, QFDs for three styles of a single product series is prepared and the results are evaluated using statistical tests.

Both primary and secondary data were collected in this study. Primary data were collected directly by measuring the glove dimensional parameters and through interviews and brainstorming sessions. Secondary data were collected from surveys, feedbacks, monthly reports, and reports generated by software used in the company.

Total glove length, palm length, palm width, finger dimensions (finger lengths-thumb, index, middle, ring, small) were collected as glove dimensional parameters. Monthly reject rates of each product style were collected for six months from the Quality Assurance (QA) Department records. These monthly reject rates were prepared by recording and summarizing the details of the daily rejected samples that are detected while the inspections of Final Release Check section of QA Department. These defects are categorized as critical, major and minor. In most cases, the customer wouldn't even notice a minor defect on a product. Hence only the Critical and major Average Outgoing Quality (AOQ) details of the three product styles were collected for 6 months.

$$AOQ = \frac{\text{Number of Defects}}{\text{Number of Samples Tested}} \times 100$$

In this study was tested using the F-test and t-test. Since t-test is used in this analysis the normality test is used to determine whether the data are following the normality test. The F-test was used to determine if there is a significant difference between the variances of the two group's data; before and after

implementing QFD. The two-sample t-test was used to determine if there was a significant difference between the means of two groups.

It is essential to find whether the product specifications are met by the products to check the progress of the study. Control charts were drawn for the glove dimensional parameters and identified whether the product specifications were met.

**Results and Discussion**

Based on normality test, the critical, major and monthly rejection rate AOQ data of three products, before implementing QFD follow normal distribution.

There were two main hypotheses tested in this study.

Hypothesis 1

$H_0$: Quality of the products is not increased after implementing QFD

$H_1$: Quality of the products is increased after implementing QFD

Hypothesis 2

$H_0$: Production efficiency is not improved after implementing QFD

$H_1$: Production efficiency is improved after implementing QFD

These hypothesis were tested using F test and t-test. The F-test was used to determine if there is a significant difference between the variances of the two group's data; before and after implementing QFD. The two-sample t-test was used to determine if there was a significant difference between the means of two groups.

The common hypothesis for the F test will be

$H_0$: Variances are equal

$H_1$: Variances are not equal

The common hypothesis for t-test in the hypotheses 1 will be,

$H_0$: Quality of the product is not increased after implementing the QFD

$H_1$: Quality of the product is increased after implementing the QFD

The common hypothesis for t-test in the hypotheses 2 will be,

$H_0$: Production efficiency of the product is not increased after implementing the QFD

$H_1$: Production efficiency of the product is increased after implementing the QFD

It is essential to find whether the product specifications are met by the products to check the progress of the study. Control charts were drawn for the glove dimensional parameters and identified whether the product specifications were met.

According to Table 1, the AOQ of three products, which have critical defects. Product B shows the variance differences before and after implementing QFD, while other two has equal variances. However based on the t test, all three products' quality has been increased after implementing QFD.

**Table 1.** P values for Critical AOQ

| Product | F-test | t-test |
|---|---|---|
| A | 0.354 | 0.007** |
| B | 0.026** | 0.013** |
| C | 0.202 | 0.014** |

**Significant value

Table 2 illustrates the AOQ of three products, which have major defects. Product C shows the variance differences before and after implementing QFD, while other two has equal variances. However based on the t test, all three products' quality has been increased after implementing QFD.

**Table 2.** P values for Major AOQ

| Product | F-test | t-test |
|---|---|---|
| A | 0.181 | 0.007** |
| B | 0.330 | 0.014** |
| C | 0.042** | 0.007** |

**Significant value

Based on the statistics for monthly rejection rate (Table 3), Product A shows the differences in rejection rate while other 2 products have no such differences. The p - value of the t-statistics of all three products is significant and hence it can reject the null hypothesis at 5% level of significance. Based on that, it can be concluded that the production efficiency has been increased after implementing the QFD.

**Table 3.** P values for Monthly Reject Rate

| Product | F-test | t-test |
|---|---|---|
| A | 0.014** | 0.006** |
| B | 0.137 | 0.006** |
| C | 0.132 | 0.025** |

**Significant value

Control charts were used to find whether the gloves met the product specifications. Control charts were drawn for the three product styles of the selected series, before and after implementing QFD. The control charts drawn for the product A are illustrated in the Figure 1.

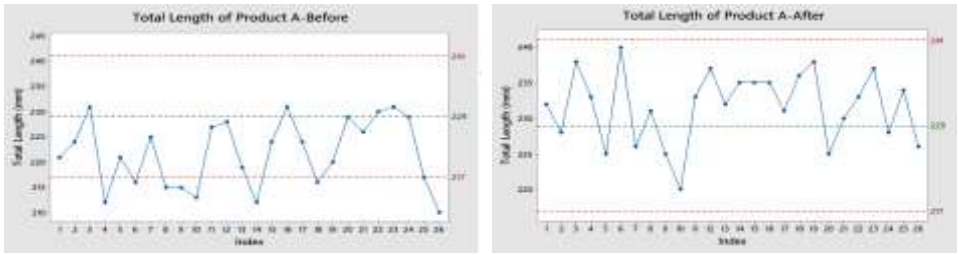


**Figure 1.** Total length of product A before and after implementing QFD

According to these control charts it is obvious that the level of meeting the product specifications is increased after implementing the QFD. The Figure 2 illustrates how product specifications of the total length of product B are met before and after implementing QFD. The number of samples that meet the specifications are increased after implementing QFD according to the above control charts.

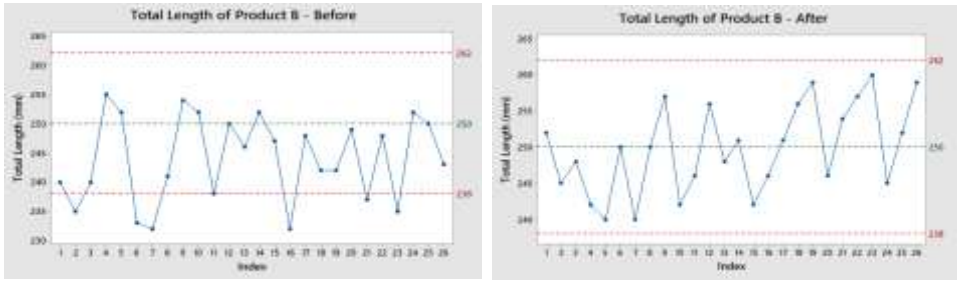


**Figure 2.** Total length of product B before and after implementing QFD

Figure 3 shows that, how product specifications of the total length of product C are met before and after implementing QFD. The number of samples that meet product specifications is increased after implementing the QFD.

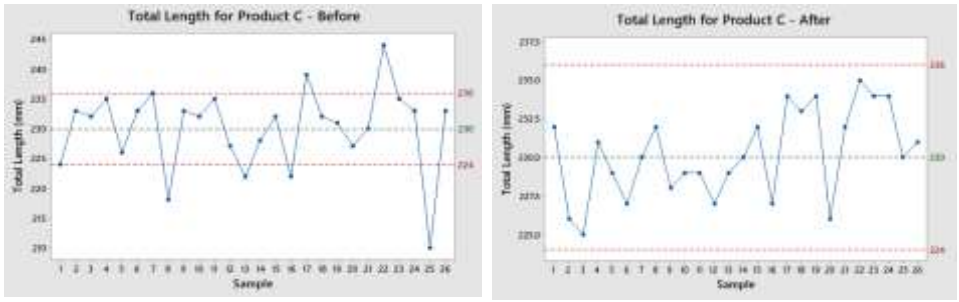


**Figure 3.** Total length of product C before and after implementing QFD

**Conclusions and Recommendations**

QFD has been regarded as a customer-oriented design tool for developing new or improved product in order to achieve higher customer satisfaction. However, achieving customer satisfaction is stated with effective recognizing, analyzing and understanding of customer requirements. In summary, this study addresses an area that is highly relevant for the glove industry where it can integrate Quality function deployment and customer requirements.

According to the above results, it can be concluded that, by transforming customer ideas into new product designs, the quality of the products can be increased. Moreover, the production efficiency of the three products was increased. QFD is a better solution for transforming customer ideas into new product designs. However, this tool could be extended, improved, and tested the future researches.

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# PHOTOCATALYTIC AND SELF-CLEANING $TiO_2$ COATING ON CLAY ROOFING TILES

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## Introduction

The long term exposure of clay roofing tiles under the real environment conditions leads to chemical and physical deterioration of both the tile surfaces and interior structure. Moisture absorbing and retaining capacity of natural clay act as a stimulus to grow microorganisms on clay roofing tiles causing biodeterioration of the tiles [1] and these microorganisms start thriving under heat and humidity which are abundant in a tropical country like Sri Lanka. This blackening of roof tile impairs the appearance of the tile aesthetically and shortens the lifetime of the tile. Present days $TiO_2$ appeared to be the widely used semiconductor photocatalytic material due to its environmentally friendly approach toward the conversion of light energy into chemical energy at mild reaction environments [2]. When irradiated with UV light, $TiO_2$ can decompose the organic pollutants present on its surface, and also, the surface turns to be superhydrophilic [2]. Due to the hydrophilicity nature of the surface of the roof tile, water can spread out instantaneously by forming a thin layer on the $TiO_2$ coated surface of the roof tile and steadily carries away dust particles from the surface while flowing.

In this research, we focused on obtaining photocatalytic mesoporous coatings using $TiO_2$ where polyethylene glycol (PEG) was used to induce porosity in the $TiO_2$ layer. Titania particles were prepared hydrothermally using titanium (iv) isopropoxide and isopropanol. The prepared UV-activated photocatalytic coatings were applied onto the clay roofing tiles and tested for their photocatalytic characteristics, hydrophilicity present after UV irradiation. The antimicrobial activity of the prepared $TiO_2$ coatings was also assessed with reference to the activity of the bacteria *Pseudomonas aeruginosa* to investigate the reduction of the biological growth (fungus) on the surface of the roof tile to reduce the chemical and physical deterioration of both the surfaces of roof tiles.

## Materials and Methods

*Preparation of roof tile samples*

The clay roofing tiles were produced in the industrial conditions at Samson Rajarata Tiles (Pvt) Ltd. Roof tiles were cut in the form of square-shaped slabs (dimensions 1.0 x 1.0 x 1.5 cm) and used as substrates for photocatalytic coating. The used raw material for the tile production was based on Illite-

Kaolinite clay material and carbonates (dolomite, calcite) that are taken from the tanks in the Anuradhapura district.

*Preparation of Titania ($TiO_2$) particles*

The preparation of titania particles hydrothermally is reported elsewhere [3]. Briefly, $TiO_2$ nanoparticles (NPs) were prepared using titanium (iv) isopropoxide (Sigma Aldrich, 97%), isopropanol (Sigma Aldrich, 99.8%), and surfactant triton X 100 (Sigma Aldrich) in the presence of dilute $HNO_3$ (Deajung, 70%). After that, a white precipitate was transferred to a Teflon container and placed in a steel autoclave at 80 $^0$C for about 20 h. Then, the white cloudy solution was filtered, dried in an electrically heated drier at a temperature of 80 $^0$C. Finally, the $TiO_2$ powder (yellowish color) obtained was transferred from the filter paper and crushed with a pestle and mortar to pulverize the agglomerates.

*Preparation of photocatalytic dispersion*

First, the $TiO_2$ aqueous solution was prepared by mixing $TiO_2$ with deionized water in a 2.5:97.5 mass ratio in a beaker with continuous magnetic stirring. Then, Polyethylene glycol (PEG) 4000 (Fisher Scientific, m.w. 4000) was added directly to the water solution in the mass% ratios of $TiO_2$/PEG 4000 of 3:0.5, 3:1.0 and 3:1.5. The solution was stirred vigorously for 1 hour and sonicated for 20 minutes. The obtained photocatalytic solution was spray-coated on to fired clay roofing tiles.

*Fabrication of $TiO_2$ layer on clay roofing tile substrate*

The stability of the suspension is essential to achieve a precise coating. Therefore, the photocatalytic solution was frequently stirred to prevent the agglomeration of particles. Three layers of the photocatalytic solution were deposited by the spray coating technique to get a stable coating on the substrate surface. Substrates were subsequently heated in an oven at 200 $^0$C for 30 minutes, (sample “A”), at 400 $^0$C for 30 minutes, (Sample “B”) finally, at 600 $^0$C for 30 minutes (Sample “C”).

*Photocatalytic behavior of the samples*

The photocatalytic activity of the roof tile samples was determined by monitoring the decomposition/decrease in methylene blue (Daejung, 97%) concentration. In this section, the effect of solution concentrations and sample heating temperatures on photocatalytic activity was investigated.

*Hydrophilicity behavior of samples*

The roof tile with the highest photocatalytic activity was chosen and tested for hydrophilicity of the coated surface. The hydrophilicity of the surfaces of the prepared samples was determined by monitoring the change in the water contact angle (WCA) with UV-irradiation time. All samples were UV-irradiated in

in-house build UV photoreactor (light intensity 0.6-0.8mW/cm$^{-2}$) for 30, 60 and 90 minutes. The distance between the UV lamp and the samples was 5.0 cm.

*The antimicrobial efficiency test*

To test antimicrobial efficiency, the standard JIS Z2801:2000 was applied using *Pseudomonas aeruginosa* (wild strain, isolated from water plants) [4]. Briefly, the Muller–Hinton agar was prepared, by mixing Muller-Hinton powder (Hardy diagnostics, Santa Maria, CA 93455 USA) with distilled water in a volume flask. Then, Muller-Hinton solution was poured into autoclaved Petri dishes and *Pseudomonas* was deposited on the surface of the agar. Finally, filter papers and piece of roof tile immersed into the photocatalytic solution was placed in the middle of the Petri dishes and one petri dish left as the reference sample. The test culture samples were left at 37 $^0$C in the oven for 24 h to grown.

## Results and Discussion

*The particle size distribution of $TiO_2$ powder*

The particle size of the $TiO_2$ that synthesis from the hydrothermal method was observed and measured using the Scanning Electron microscope (Zeiss EVO LS15) and DLS technique (Cilas Nano DS dual light scattering particle size analyzer). SEM images are shown in Figure 1 below. $TiO_2$ particle size in colloidal dispersion was around 50-100 nm.

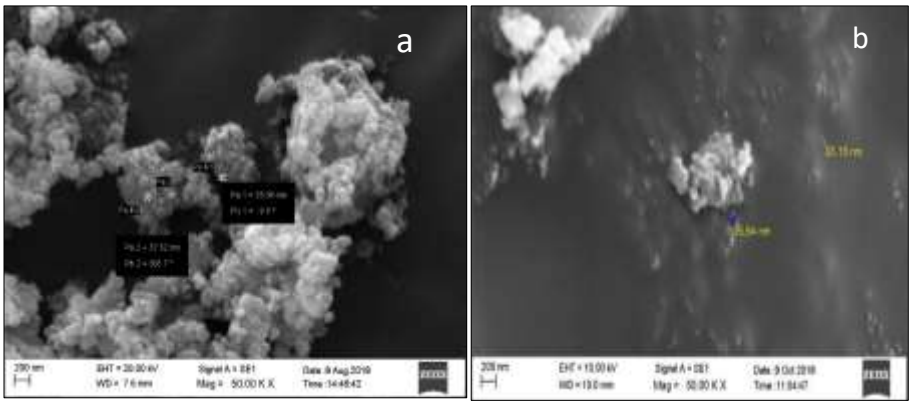


**Figure 1.** $TiO_2$ particle size (a) without triton X-100 (b) with triton X-100

*Photocatalytic behavior of the samples*

PEG has been used as a pore-generating agent in preparing porous $TiO_2$ films because the porosity of the $TiO_2$ film increases the active catalytic surface [5]. The following figures show the photocatalytic activity of the samples, by varying the amounts of the PEG masses that incorporated in photocatalytic solution and roof tile samples burned at 200 $^0$C and 400 $^0$C.

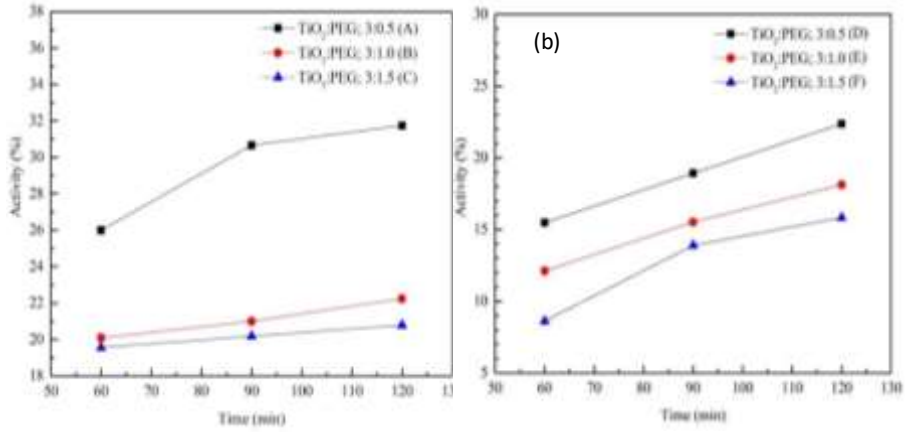


**Figure 2.** (a) Photocatalytic activity vs UV irradiation time for 200 $^0$C (b) photocatalytic activity vs UV irradiation time for 400 $^0$C

Overall, several conclusions can be drawn from the above figure 2. These results demonstrate the fact that the sample with heat treatment at 200 $^0$C was the most photocatalytically active than the sample with 400 $^0$C. Also in the 200 $^0$C sample set, the sample with $TiO_2$: PEG 3:0.5 shows the highest activity than the other two samples, and this trend is again observed for 400 $^0$C sample set. The fact behind the 0.5 g PEG to be the most active sample of all heat treatment categories is due to the thin layer of coating of PEG, hence, more Methylene blue molecules were probably adsorbed. The addition of more PEG weight may have blocked the active catalyst sites by the formation of the intermediate products and consequently, the photocatalytic activity of the samples with thick PEG coatings was decreased. Therefore, $TiO_2$: PEG 3.05 was chosen as the best $TiO_2$: PEG combination and was tested for hydrophilicity and antimicrobial efficiency.

*Hydrophilicity behavior of samples*

The results indicate that the WCA of the sample B and C roof tile was not significantly changed after UV irradiation, but the fired tiles with sample A showed a dramatic decrease in CA as a function of UV irradiation time (Figure 3).

From these results, it was determined that approximately 100 min is sufficient illumination time to give a highly hydrophilic surface in both cases. It has been reported that the increase in hydrophilicity is due to the photoreduction of the $Ti^{+4}$ sites to the $Ti^{+3}$ of the surfaces after UV illumination [2]. According to the results, one could speculate that the smaller contact angle of samples B and C

compared to sample A before UV-irradiation is due to the lower presence of $Ti^{+4}$ on the surface of the thin layer.

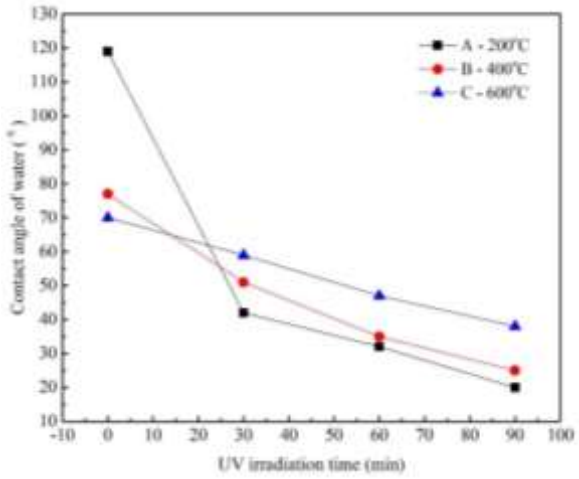


**Figure 3.** The contact angle of water vs UV irradiation time

*The antimicrobial efficiency test*

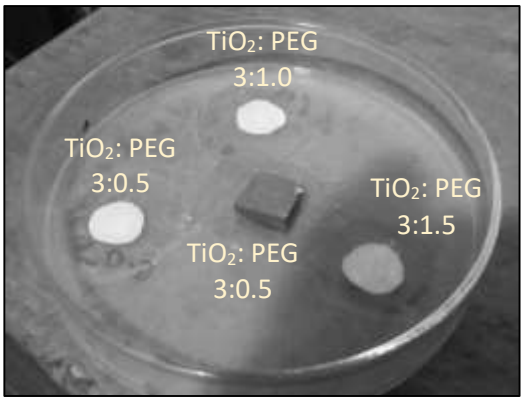


**Figure 4.** The inhabitation zones of *Pseudomonas aeruginosa* after 24 h on Müller-Hinton agar

Figure 4 above shows the inhabitation zones of *Pseudomonas aeruginosa* after 24 h of UV irradiation on Müller-Hinton agar with a deposit of different concentrations of $TiO_2$ photocatalytic solution and piece of coated roof tile sample. The diameters of all inhabitation zones were approximately equal to the 1.4 cm because all 3 filter papers have the same $TiO_2$ concentration but different PEG masses. Moreover, we can further conclude that PEG does not have any antimicrobial properties and $TiO_2$ become more antimicrobial with the

increasing UV irradiation time. The irradiation of UV light on $TiO_2$ generates hydro radicals [2]. These hydro radicals can act as scavengers, they can decompose these organic compounds in bacteria into the water and $CO_2$.

**Conclusions and Recommendations**

It was confirmed in this study that the $TiO_2$ coated clay roof tiles show good photocatalytic activity with high hydrophilicity and antimicrobial activity. All samples became hydrophilic with the presence of Ti–O species on the surface, upon UV irradiation. Moreover, the photocatalytic activity was increased with a thin layer of $TiO_2$ (0.5 g PEG mass), after 120 min of UV-irradiation treatment compared to the already reached activity plateau of the sample with a thick layer (1.5 g PEG mass). Besides, photocatalytic activity was higher at 200 $^0$C and decreased with increasing temperature of heat treatment to 400 $^0$C due to $TiO_2$ transition from anatase to rutile starts around 400 $^0$C [6].

**Acknowledgment**

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# OPTOELECTRONIC DISPLACEMENT SENSOR AND ENCODER BASED HYBRID APPROACH FOR MOBILE ROBOT ODOMETRY

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## Introduction

With modern technology evolution, "Mobile Robots" become very popular due to their autonomous or non-autonomous ability to move within its environment [1]. Identifying the position and calculating travelled distance is a significant aspect of mobile robots. A rotary encoder is a displacement sensor which is commonly used in mobile robots. It is an electro-mechanical device that converts the angular position to an analog or digital output signal for measuring robot's displacement or velocity [2, 3]. However, this approach tends to face difficulties such as systematic and non-systematic errors (slipping, crawling, unequal wheel diameters and imprecisely measured wheel distance) [3]. Due to these reasons, another method was introduced to the mobile robot field; a computer mouse sensor-based position sensing [2]. Thereafter, rotary encoders were replaced with mouse sensors to produce better results [4]. Even so mouse sensors provided higher sensitivity; their working nature required controlled surface properties to work well [1, 3]. In this research work, it was aimed to develop a position sensing and travelled distance measuring approach by combining rotary encoder and optical mouse sensor. Further, the Internet of Things (IoT) capability to produce navigation data in real-time is tested to adopt more flexible operation monitoring technique. The accuracy and robustness of the mobile robot were investigated with a pre-planned test and recorded data were used to optimize a position sensing protocol.

## Materials and Methods

General-purpose four encoder motors and a gaming mouse was used as the displacement sensors. The robot's building structure was developed under two hardware layers, as shown in Figure 1(a). A Smart LinkIt 7688 Duo board was used as the central controller. The L298n IC-based motor driver circuit was used to drive the motors. The portable power supply was used with a step-down voltage regulator circuit to power the mobile robot. The constructed robot is

weight around 1.8 kg and has a dimension of 10, 19 and 23 cm for height, width and length, respectively.

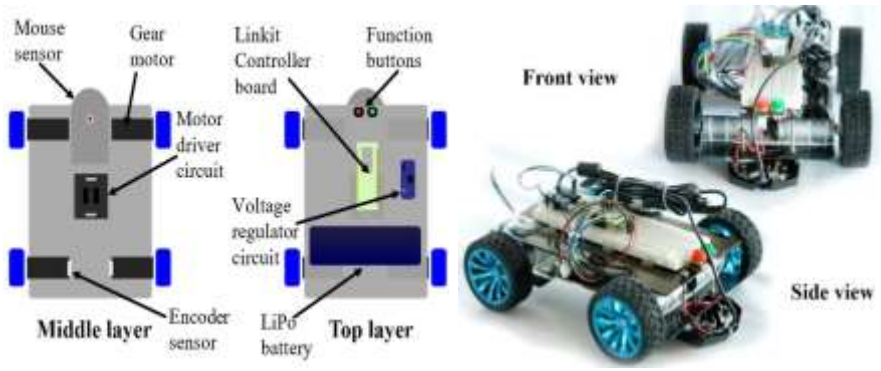


**Figure 1.** Physical view of the robot (a) fully assembled mobile robot diagram (b) Fully assembled mobile robot images

The developed mobile robot's control flow structure and its respective operations were shown in Figure 2. In the constructed mobile robot system, the ATmega32u4 microcontroller (MCU) was used to read raw encoder data and simultaneously operate the mobile robot wheels. The obtained encoder data were fed to the microprocessor (MPU) for further processing by using an inbuilt UART connection. Since MPU had a mini USB port and a Linux distribution to control board components, the native C based system application programming model was selected as the central controller firmware language [5]. Through SSH (Secure Socket Shell) the programmed package files were installed and configured to read and manipulate the mouse sensor data.

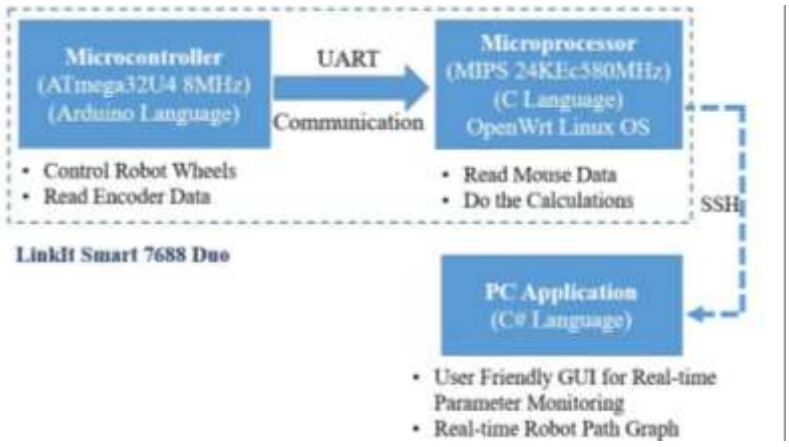


**Figure 2.** Control flow structure of the mobile robot

Based on the mouse sensor data, a stream of X and Y coordinates obtained in real-time. To calculate the travel distance X, Y coordinates were used. The equation (1) is used to calculate the distance between two points. Where D is the distance, dx and dy is the difference between the X and Y-coordinates.

$$D = \sqrt{dx^2 + dy^2} \tag{1}$$

Inside the main firmware, the mouse data were collected with respect to corresponding encoder data. Both sensor data were then used to calculate the travelled distance. Finally, calculated data values were communicated to a PC through SSH. A PC application was developed by using C# language with the ability to capturing real-time mobile robot data and visualized them graphically and numerically. All the data can be found in the GUI interface as shown in Figure 3. In addition to the local network connectivity via SSH (using Wi-Fi), real-time data can be access through the Internet with proper DNS configurations. Hence, the developed mobile robot can be used as an IoT device to monitor or control data and operations.

To develop a proper position sensing protocol for the mobile robot, a test (test#1) was designed to investigate the sensor data. By using this test, it is expected to observe each sensors affinity to follow actual distance measurements. The test was to travel the robot for a pre-defined period in a linear path without any kind of pre-programmed turns. This was performed repeatedly to test robots' robustness of data. The actual distance, the mouse sensor-based distance, and encoder sensor-based travelled distance was recorded. For measuring exact length, a calibrated meter ruler was used.

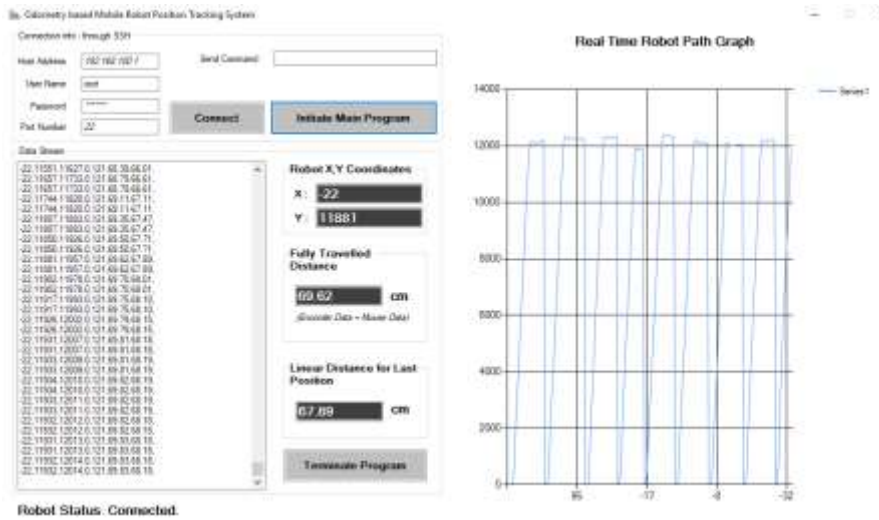


**Figure 3.** GUI interface with real-time data plotting graph

**Results and Discussion**

Figure 4 shows the raw data plotted against twenty-five (25) number of rounds of test#1. With the small variations in actual distance, it is clear that encoder and mouse raw data have an affinity to follow the actual distance. During our initial experiments, we have changed the mouse sensor quite a few times to check their reliability. The observations show that low-cost general-purpose mouse tends to miss data points at a longer distance than the higher-cost mouse that has higher DPI, which was matched with several recent research works [3]. The travelled distance calculated by combining encoder and mouse sensor data. The mean approach was employed at first, and accuracy was calculated using equation 02.

$$[\text{Traveled Distance}]_{\text{M}} = \frac{\left(\text{M\_distance}/_{\text{a}}\right)+\left(\text{E\_distance}/_{\text{b}}\right)}{2} \qquad (2)$$

Where $[\text{Traveled Distance}]_{\text{M}}$ is calibrated travelled distance using mean approach, M_distance is mouse sensor raw data, E_distnace is encoder sensor raw data and a, b are the calibration values as follows,

$$a = \frac{\sum_{i=1}^{n} x_i}{n}, \quad b = \frac{\sum_{i=1}^{n} y_i}{n} \qquad (3,4)$$

Where *a* and *b* median value obtained from mouse sensors' and encoders' data (n = number of rounds), $x_i$ and $y_i$ values were their rounds number.

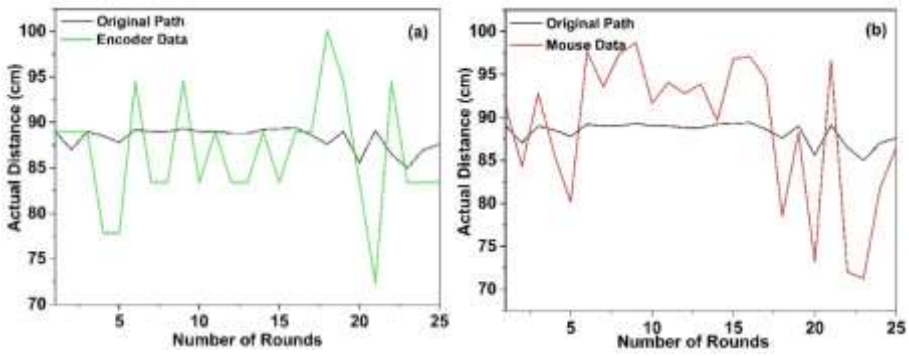


**Figure 4.** Actual distance comparison for twenty-five test rounds with (a) raw encoder distance data and (b) raw mouse sensor distance data

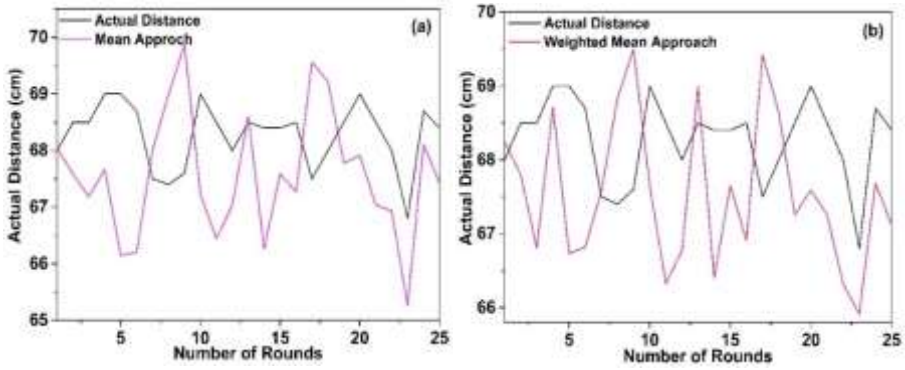


**Figure 5.** Comparison data for twenty-five test rounds with (a) mean approached and (b) weighted mean approached

According to the equation 02, the comparison curves were shown in Figure 5. The mean approach curve indicates a smoother pattern toward actual distance than raw data in Figure 4. In Figure 5(a), the mean approach error was calculated approximately as +0.5 and -1.1 cm. The error calculation was done depending on the total length traveled. Furthermore, to gain more accuracy, the weighted mean approach was used, and equation 02 was modified as follows. C and D are the weighted constants.

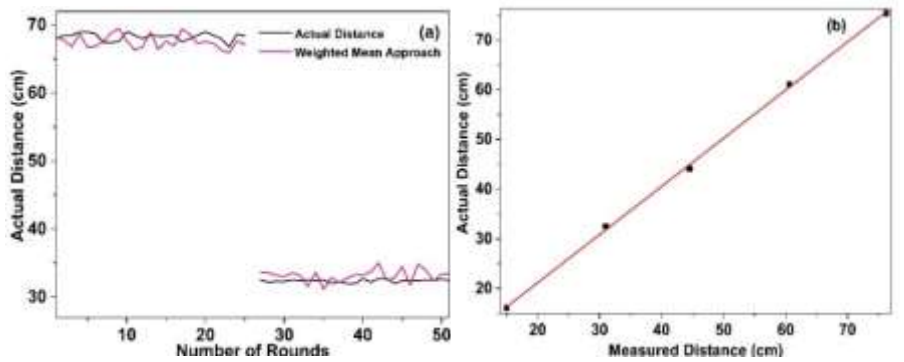


**Figure 6.** (a) Comparison of two different distance values with respect to actual and weighted mean approach distance (b) Linearity over distance plotted against five different distances.

$$[\text{Travelrd Distance}]_{\text{WM}} = \frac{\left(\left(\text{M\_distance}/_{\text{a}}\right)*\text{C}\right)+\left(\left(\text{E\_distance}/_{\text{b}}\right)*\text{D}\right)}{2} \qquad (5)$$

The weighted mean approach error was calculated toward actual distance and found approximately +0.39 and -0.76 cm. The error calculation was done depending on the total length traveled. As per the calculated results, it was clear that the weighted mean approach has a reduced error. To analyze the robot sensitivity, a comparison graph was obtained by performing test#1 for two different distances. The respective graph was shown in figure 6(a). The graph is a comparison between actual distance and weighted mean distance. The results show that the travel distance follows the actual distance without any significant distortion. Moreover, linearity over distance was further investigated for five values as shown in Figure 6(b), and its fitted line depicts excellent linearity. This result suggests that the robot is capable of giving accurate travelled distances over different values. However, more experiments will be performed to understand the sensors' performance over nonlinear paths and ability to use more accurate sensor data combining algorithms such as Kalman filtering.

**Conclusions and Recommendations**

In this paper, the feasibility of combining the optical mouse sensor and an encoder sensor for accurate odometry was presented. The system provides a good sensing capability rather than calculating distance using one kind of sensor. The weighted mean approach provided low uncertainty values as observed, +0.39 and -0.76 cm, respectively. The proposed system shows encouraging and robust results by overcoming each sensor's drawback while producing stable data over time. Moreover, the IoT capability in the developed method can be a useful key to address the current demand for technologically advanced robotic navigations.

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# DISSOLVED CONTAMINANT TRANSPORT IN HOMOGENEOUS AND DIFFERENTLY-LAYERED GEOSYSTEMS: AN EXPERIMENTAL AND NUMERICAL INVESTIGATION

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## Introduction

Groundwater has been a major source of water supply in Sri Lanka. About 80% of the rural domestic water supply needs are met from groundwater by means of dug wells and tube wells (NWSDB & WRB). Groundwater resources are under increasing threat due to the fate and movement of the dissolved contaminant in soils. Saline water intrusion is the major issue in Sri Lanka, especially in Jaffna peninsula and southern region of Sri Lanka (along the Benthera river). Soil-texture and soil-heterogeneous are the main physical controls which can potentially affect the advection, dispersion, diffusion and retention of the contaminant, and hence the migration of dissolved contaminants in subsurface soil. A key to the management of groundwater is the ability to model the movement of dissolved contaminants in the subsurface environment. Although several experimental studies as well as analytical investigations [1, 2, 3] have been devoted to measure and model the transport behaviors of the dissolved contaminant in differently-layered soils, numerical studies on subsurface contaminant migration have not been adequately studied. Most analytical solutions fail to provide acceptable results in the case of complex boundary conditions and for heterogeneous and anisotropic aquifers. In such cases, numerical solutions will provide a better alternative to the modelling of contaminant transport through porous media [4].

This study investigated dissolved contaminant transport in homogeneous and differently-layered porous media. Fine-grained sand from a beachside in Jaffna Peninsula and coarse sand near a river bed from southern region of Sri Lanka were sampled for this study. A controlled laboratory experiment was conducted in a bench-scale 2-D tank to measure contaminant (0.1M NaCl coloured contaminant) transport along the depth with time. Contaminant concentration was measured at selected sampling ports at regular time intervals and the movement of the color dye was observed in parallel to visualize the contaminant migration patterns with time. The experiment results were compared with a numerical result of COMSOL, multiphsyics classical advection-diffusion/dispersion modeling framework to prove the applicability of COMSOL to analysis subsurface contaminant concentration and migration in the absence of direct measurements.

## Materials and Methods

*Experimental analysis*

A medium bench-scale 8-mm thick Plexiglass 2-D tank of dimensions 1 m (length) x 0.3 m (depth) x 0.1 m (width) was fabricated. Fine sand (to mimic homogeneous soil system) and different layers of fine and coarse sands (to mimic layered-soil system) were individually wet-packed in the tank (Figure. 1). Physical properties of coarse and fine sand are presented in Table 1. The dissolved contaminant (0.1M NaCl) was injected at the top-mid surface at a gravel-packed short section as shown in Figure 1. The contaminant was coloured to visually investigate the migration pathways with time. The migration patterns of the moving coloured contaminant with time were examined through the photographs taken in different time intervals. Simultaneously, contaminated water samples (80ml) were extracted from sampling ports (Figure. 1) to measure the electrical conductivity of the sample. Measured electrical conductivity was used to estimate the contaminant (NaCl) concentration by following the implicit analogy developed by Porro [5] as presented below:

$$\frac{C(t)}{C_0} = \frac{EC(t)}{EC_0}$$

where $\frac{C(t)}{C_0}$ is relative concentration (C (mols) and $C_o$ (mols) are NaCl concentrations of the solution at time t and $t_o$ (t=0), respectively) and EC (mS/m) and $EC_o$(mS/m) are corresponding electrical conductivity.

**Table 1.** Physical properties of investigated soil.

| Soil type | Bulk density | Particle density | Total porosity | Intrinsic permeability (k)₱ |
|---|---|---|---|---|
| | $g/cm^{-3}$ | $g/cm^{-3}$ | $cm^3\ cm^{-3}$ | m2 |
| Fine-grained | 1.6 | 2.65 | 0.4 | 2*10-11 |
| Coarse-grained | 1.47 | 2.65 | 0.44 | $2.29*10^{-8}$ |

₱ Hydraulic conductivity was measured by constent head method

*Numerical analysis*

COMSOL (version 4.4), geophysics module was used for the numerical simulation of contaminant transport with time. A 2-D computational domain (x and y axis of the domain represent the width and height of the experimental tank, respectively) was created for this simulation (Figure. 1). We assumed the material properties of the sand are homogeneous and isotropic. The vertical lines in x-axis represent a symmetry boundary with a mirror image of the cross-section extending beyond it. No flow condition was set across the domain edges. High salt concentration (0.1M NaCl) was set at the mid-section of the

upper boundary (width is 20 cm, same as in the experimental setup) whereas salt concentration was set to zero along the lower boundary. The governing equations involved in this numerical simulation are as follows:

Fluid flow can describe in this simulation using Darcy's law,

$$\frac{\partial \varepsilon \rho}{\partial t} + \nabla . \rho u = 0 \qquad (2)$$

where, ρ is the water density (kg/m^3), ε is the porosity, u is the vector of directional seepage rates, also known as Darcy velocity. It can be represented as bellow:

$$u = {}^{k}/_{\mu} (\nabla \mathrm{p} + \rho \mathrm{g} \nabla \mathrm{D}) \qquad (3)$$

where k is permeability ($m^2$), μ is the fluid's dynamic viscosity (Pa.s), p is the fluids pressure (Pa), D represents y co-ordinate of the domain. Fluid density can describe in this simulation as follows:

$$\rho = \rho_o + \beta_c = \rho_o \left( \frac{\rho_c - \rho_o}{c_s - c_o} \right) C \qquad (4)$$

where, $C_o$ and $C_s$ are the normalized salt concentrations of pristine and salty water, respectively and $\rho_o$ is the density of the pristine water. In this simulation, the governing equation are conservative form of the solute transport interface

$$\frac{\partial \theta_s c}{\partial t} + \Delta . cu - \Delta . \theta_s \tau D_L \Delta_c = 0 \qquad (5)$$

where $D_L$ is the fluid's diffusion coefficient ($m^2$/d), $\theta_s$ is the fluid's volume fraction (porosity), *c* is the salt concentration (kg/$m^3$), and u is the Darcy velocity (m/s).

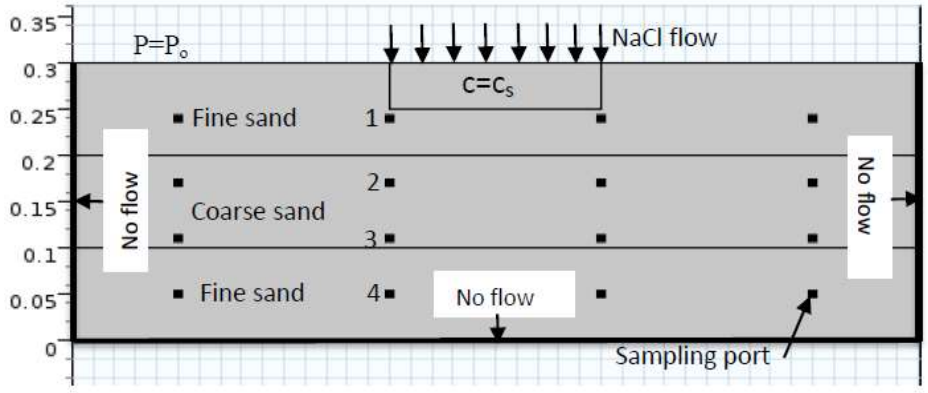


**Figure 1**. 2-D computational domain for the numerical simulation. Dimensions are same as the experimental tank.

*Statistical validation*

The proposed model was compared and tested with the measured contaminant (NaCl) concentration data for the general accuracy using RMSE:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(d_i)^2} \quad (6)$$

where *di* is the difference between the measured and simulated value of NaCl concentration and *n* is the number of measurements in the data set.

**Results and Discussion**

A uniform and regular distribution of contaminant transport is observed in homogeneous soil system (Figure 2 (a)) whereas irregular distribution of contaminant transport is observed in the layered-soil system (Figure 3(a)). The high density of NaCl (1200 kg/m$^3$) as compared to the pure surrounding water (1000 kg/m$^3$) causes density-driven advective flow, leading to a downward-bulging contaminant flow in both homogeneous and layered-soil system. The lateral and downward movements, however, are only diffusion-controlled and diffusive movement is virtually the same in all directions for homogeneous soil system, but it is markedly different in the layered-soil system due to the different pore connectivity at the textural interface, and hence constrains the contaminant movement.

Figure 2(b) and Figure 3(b) show the numerical results of contaminant transport with time in the homogeneous and layered soil system, respectively. In the homogeneous soil system, the numerical solution matches extremely well with the experimental result. Contaminant flow is faster in numerical result than that of in the experimental result in layerd-soil system. It may be due to the change in porosity of lower layer fine sand during the packing.

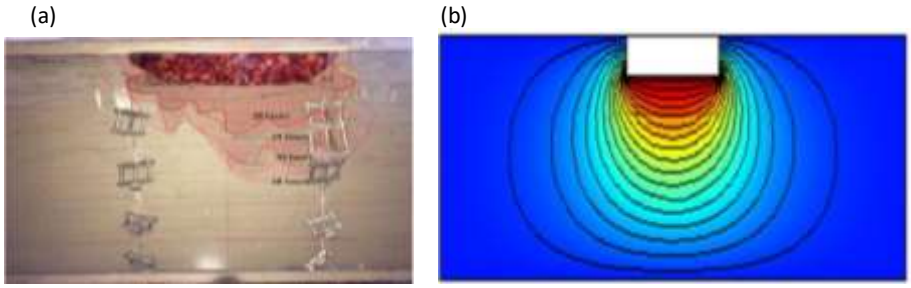


**Figure 2**. Experimental (a) and numerical (b) results of the contaminant migration in homogeneous soil system (fine-sand)

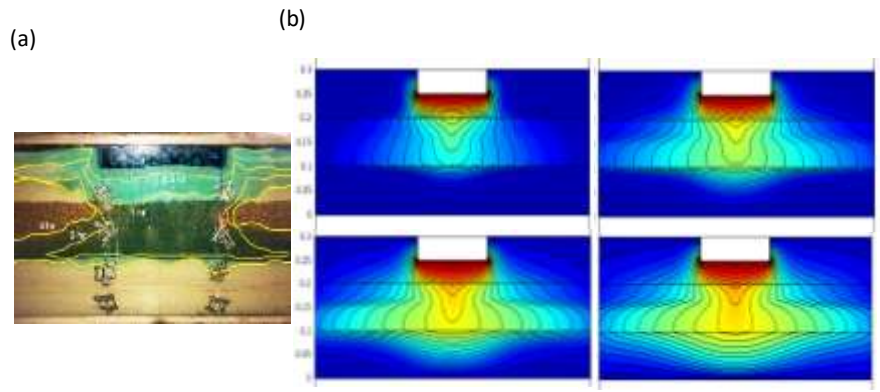


**Figure 3**. Experimental (a) and numerical (b) results of the contaminant migration in layered-soil system (fine-coarse-sand)

Figure 4 shows the comparison of experimental and numerical results of contaminant transport in layerd-soil system along on of the contaminant extraction points. Although some differences exist between the numerical results and the experiment result in layered soil, the magnitude of the error was acceptable, implying the applicability of COMSOL, geophysics numerical module to estimate subsurface contaminant transport.

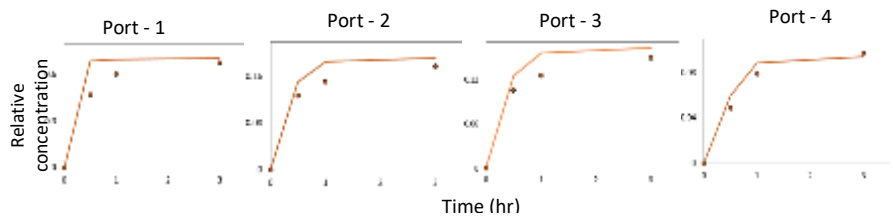


**Figure 4**. Comparison of experimental (solid circle) and the numerical (solid line) results of relative concentration with time along the sample extraction ports 1, 2, 3 and 4 (see Figure.1) in layerd-soil system.

## Conclusions and Recommendations

Experimental results of the contaminant transport in homogeneous and layered soil-system were compared with those from numerical results. Contaminant transport is distinctly different in homogeneous and layerd-soil system Furthermore, the flow has barely penetrated to the underlying fine layer until the contaminant was spread across the entire coarse sand domain, suggesting that in natural soils systems, the presence of a fine textured layer above the groundwater table can potentially act as a protective contaminant barrier, protecting groundwater from dissolved contaminants. Notably, measured contaminant concentration could be adequately numerically simulated in both

homogeneous and heterogeneous soil system, recommends the applicability of COMSOL geophysics numerical module to estimate subsurface contaminant migration in the absence of direct concentration measurements.

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# FOCUS AREA

## Energy

# OPTIMUM DESIGN IMPROVEMENTS FOR LIVE LINE INSPECTION ROBOT FOR 33 kV TOWER LINES

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## Introduction

Ceylon Electricity Board (CEB) is the major electricity provider in Sri Lanka which is authorized for generation, transmission and most of the distribution of electricity. Underground cables and overhead lines are the main types where each transmission line types have its own advantages and disadvantages and line is selected considering technical and economic factors. Ceylon Electricity Board transmits power mainly using 33 kV tower line networks and it covers 40% of the distribution network. Most of these distribution tower lines are aged more than twenty-five years. They can be damaged by oxides and corrosions. Repetition of expansion and contraction of the transmission line due to temperature changes induce fatigue stress to the line mainly due to the aging of the material. Mainly 33 kV lines must be in a good condition because this affects all consumer reliability to supply reliable and uninterruptible power. Therefore, it is highly important to maintain reliability of 33 kV overhead lines.

At presently 33kV tower line maintenance is done by linemen who engaged manually for inspection of line faults, clearance etc under live line condition [1]. Most of the time workers need to attend maintenance on electrically energized (live) power lines. They may perform a number of tasks associated with power lines, including inspecting damages of power lines. Line workers, especially those who deal with live electrical apparatus is performed very dangerous job. Therefore, CEB is the responsible party for the labor who carries out the maintenance since there is a possible risk.

In developed countries, most of the maintenance and inspection of live power lines are done by robots [2]. In Sri Lanka, still this technology is not applied for line maintenance and several initiatives have been developed in experimental scale. To avoid above-mentioned problems in live line maintenance, it has been introduced line inspection robot prototype called “LIVE LINE INSPECTION ROBORT FOR 33 kV TOWER LINES” by the Open University student for his undergraduate project [3]. This robot is running on single line cables and control via remote controller. Therefore, this robot can take closer visual images of the energized live line without risk. Using this robot inspection conductor mid-point and strands status, disk insulator status like corrosion of pins can be observed.

However, there are several drawbacks identified in the existing structure of the robot.

- Heavy structure cannot be applied to the line because line will sag
- Obstacle passing method might not be working 100% accurately
- Camera system cannot cover 360 degrees of conductor

Major problem in the existing robot is the weight of the robot. As the solution, new system is designed to minimize above mentioned drawbacks of the original robot and lead to a better outcome. Using lightweight material and redesigning of simple structure the newly designed robot can be improved. Improvement of inspection capabilities using new cameras will be integrated to the new design. Instead, normal cameras infrared obstacle detection sensors will be incorporated so that suspension clamps will be recognized. Obstacle avoidance will be done by two insulated robot arms and two wire tongs. High capacity battery will be powered two main boards and it will be helped to extend performing time.

**Materials and Methods**

New structure for live line inspection robot is designed using lightweight materials and motors to reduce overall weight of the system. Instead of high-power motors, medium power dc motors with gear unit is used to reduce weight. Robot's arms are designed to hold the total weight of the system and keeps balance the system while moving on the wire. Each arm has dedicated work to achieve to complete inspection process. Wiretoung units are helps to hold system while arms avoiding the obstacles. Three cameras mounted on left arm are used to inspect 360 degree of the conductor. Circuits and battery are included inside the carriage.

*Block diagram for robot design*

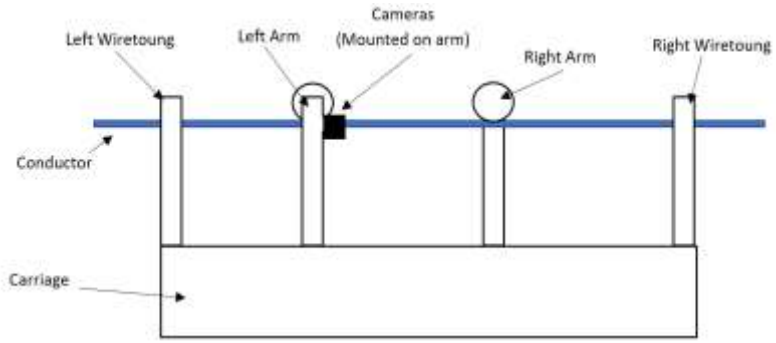


**Figure 1**. Block diagram of robot

Figure 1 shows the Block diagram of the robot and figure 2 shows all the components of the block diagram.

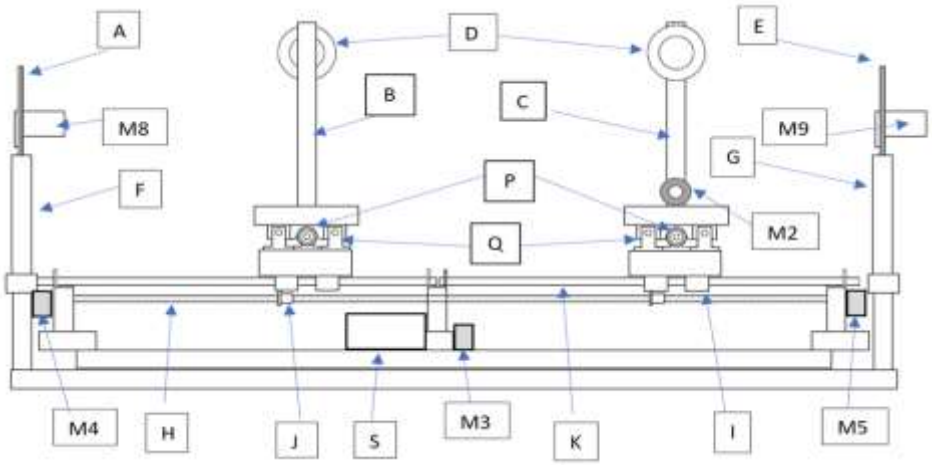


**Figure 2.** Side View of the robot

A- Left wiretoung
B- Left Arm
C- Right Arm
D- Teflon insulated Wheels
E- Right wiretoung
F- Left Wiretoung base
G- Right wiretoung base
H- X - axis Unit movement guide thread bar
I- Linear Ball Bearing
J- T Type Brass lead Screw Nut
K- X-axis Guide Runner
L- Y- axis Unit movement guide thread bar
M1- 12V 5A dc motor
M2- 12V 9A dc motor
M3- 12V 9A dc motor
M4- 4 Wire Stepper Motor
M5- 4 Wire Stepper Motor
M6- 4 Wire Stepper Motor
M7- 4 Wire Stepper Motor
M8- Metal Gear 2BB Torque RC Servo
M9- Metal Gear 2BB Torque RC Servo
N- Z-axis Guide Runner
O- Z- axis Unit movement guide thread bar
P- Pillow block bearing
Q- Linear shaft guide support
R- Y-axis Guide Runner
S- 12V Li-ion Battery
T-IR (INFRARED) Obstacle detection sens

*Control process of the robot*

Overall system is controlled under two main boards, Raspberry pi 3 and Ardiuno mega. Raspberry pi 3 is used as the main board which does communication in the robot. Wireless communication between ground and robot, live video transmission, serial communication between main board and Arduino board are major duties of this control board. Arduino mega board is used to control motors, Control PIDs and control sensors [5].

All motor drives used encoders and PID control because of its simplicity and precision. The encoder acts as a feedback from the motor, it is connected to the microcontroller (Raspberry pi-3) for further processing. With the use of Raspberry pi-3, l298N and Optical Quadrature Encoder can drive the dc motor at desired speed having a feedback loop which is taken to control motor errors, speed and performance. Figure 3 shows the control process of the robot.

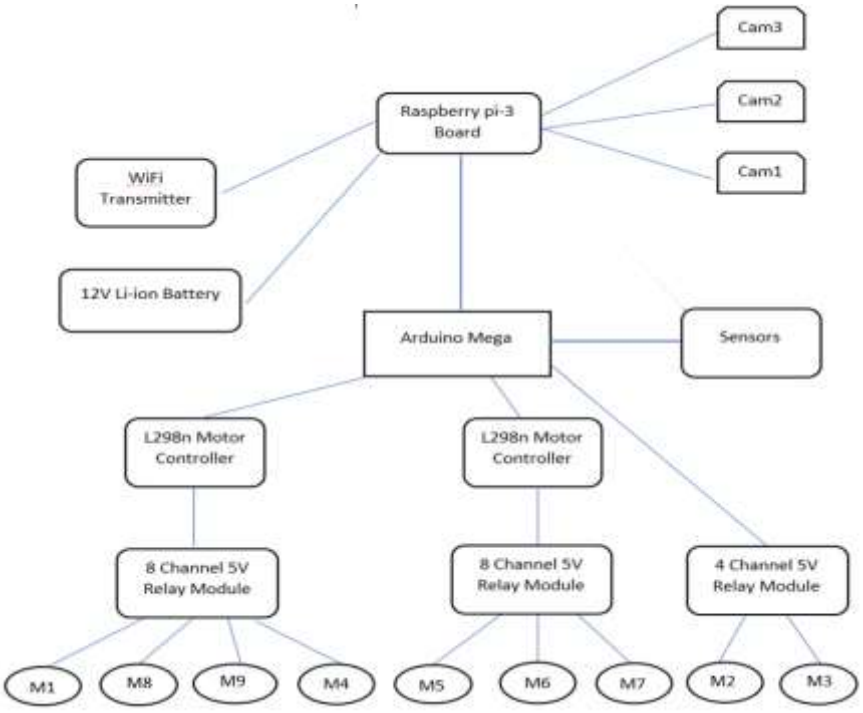


**Figure 3.** Control process of each component

*Working Mechanism of Robot*

The Robot can be moved in the X, Y, and Z directions using the movement control system in the remote controller. X-axis movements guide the robot arm unit Platform backward and forward. Y-axis movements move the robot arm left

and right. Z-axis movement moves the entire system (x-axis units and y-axis units) up and down. There are two main sections in the Overall system, main unit with robot arms and wiretoung unit. There are nine motors in overall system and each one has dedicated task. The main two motors provide motion to systems. Another four stepper motors are used to move robot arm along X and Y axis and another two servo motors are used to move wiretoung. It can be said that the motors weight is also a considerable parameter when reducing the overall machine weight.

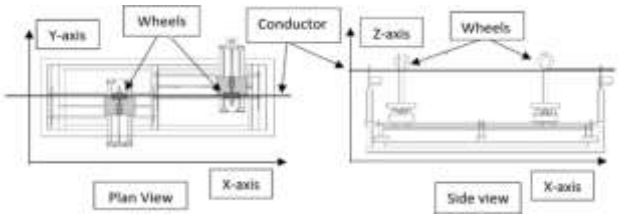


**Figure 4.** Axis of overall system

The wiretoung unit can be moved along the main structure through screw bar and it will hang the system till the main unit passing through obstacle. Until first arm passing through obstacle second arm is used to ensure stability and assist in the overtaking of obstacles in the lines. IR (INFRARED) obstacle detection sensor is used to identify obstacles and activate the obstacle passing unit. It can also be activated the obstacle passing unit via a remote controller because it is unable to identify little obstacles by the sensor itself.

*Design of the arms and wiretoungs*

Arms and wiretoungs are the main components in this robot. Each component has dedicated task. The right arm of the Robot is doing the basic function of the robot's moving mechanism. The metal gear wheel that attached to the end of the axle transmits the power from DC motor using timing chain. The Teflon wheel is rounded by a rubber grip ring to build enough friction to move. Left arm which is shown in Figure 5 is used to attach the camera setup and distance measuring encoder.

*Ground control unit (User interface)*

Virtual remote controller is designed to control the robot. There are two modes in the controller, manual mode and automatic mode. Every motor can manually switch on/off separately. Obstacle can be avoided automatically using automatic mode. Dashboard will be any device which can connect to internet like PC, laptop or mobile phone with monitor. Figure 6 shows the graphical interface of the remote controller of the robot using PC.

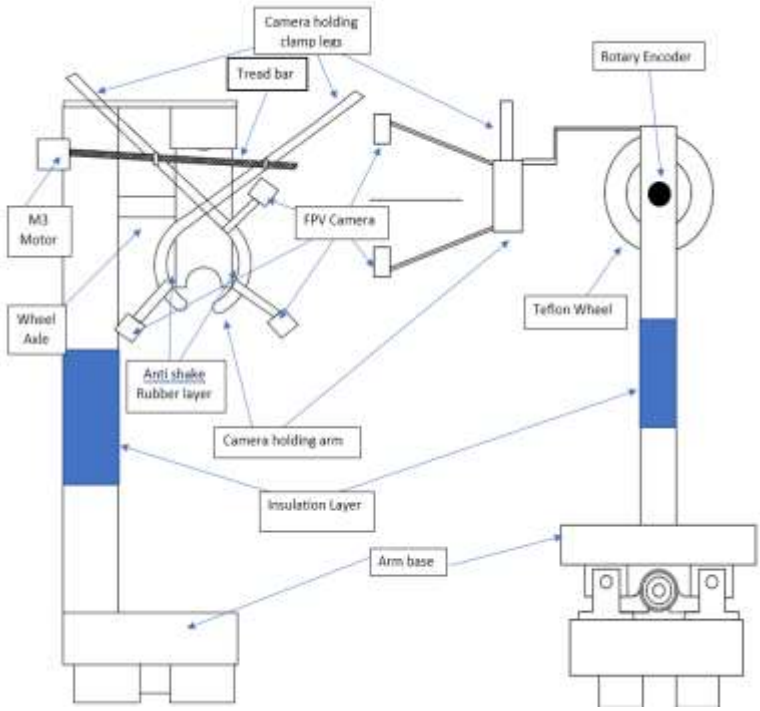


**Figure 5.** Left arm

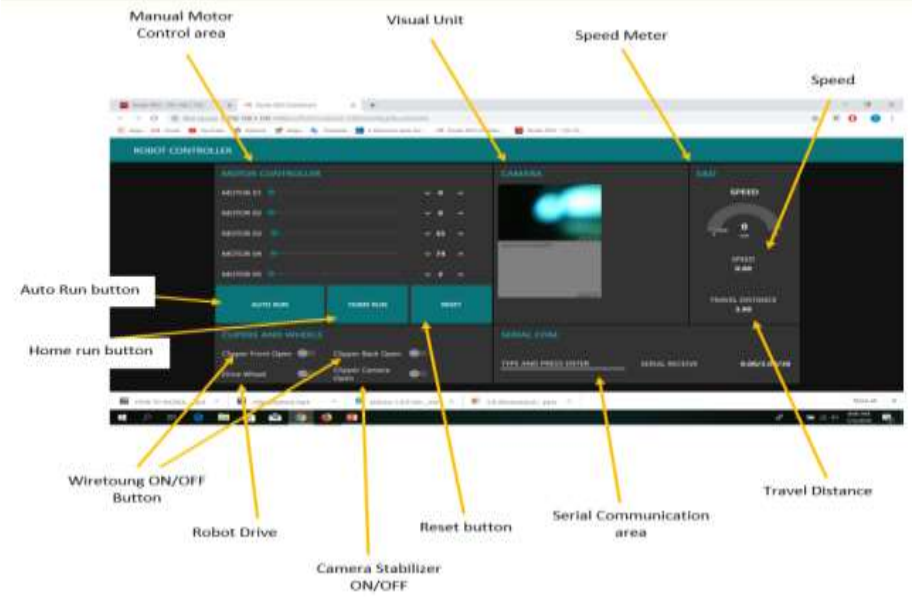


**Figure 6.** Ground control unit

**Results and Discussion**

This newly designed robot is first tested in conductor without live supply. It was proven that mechanical design of the robot is achieved as given in the objectives. Also robot successfully sent the inspection data to the ground controlling unit. Figure 7 and 8 shows the test run conditions and figure 9 shows the monitoring of ground controlling unit while robot is running on the test run.

**Figure 7.** Robot moving on line (test run)

**Figure 8.** Testing of wiretoung

From Figure 9 it can be clearly seen the conductor condition on monitor of ground controlling unit and it covers around 360 degrees. Aluminum is selected as basic material to make the main structure. Aluminum is a light weight, strong material and its density is 65% of lower than steel. This durable material has high corrosion resistant properties and it is a nonmagnetic material. Major disadvantages of aluminum are low yield strength compared to steel and it requires special processes to be welded. Because of that some parts were made using steel to maintain strength.

Main two wheels were made by Teflon. Teflon has high dielectric strength and extremely low conductivity properties. Its melting point is approximately 327°C and has Chemical Resistance. The main disadvantage is its low friction. Therefore, there should be a rubber coating to increase the friction of the wheels. Using a 3D printer, this machine could be done less weight with fine finished plastic parts. Lack of modern machineries, it is very hard to get it done accurately. The most critical requirement for precision was that the axes are aligned at exact right angles to each other. If not aligned, the work done by this robot will also not be precise and aligned.

When the two relay modules were powered by the Arduino board, the power was insufficient. Therefore, finally a regulator was used to power them both. When arms move simultaneously, the moving process is become slow because

single core processor is unable of multitasking. The Camera frame rate is little low when processing and transmitting three camera videos simultaneously.

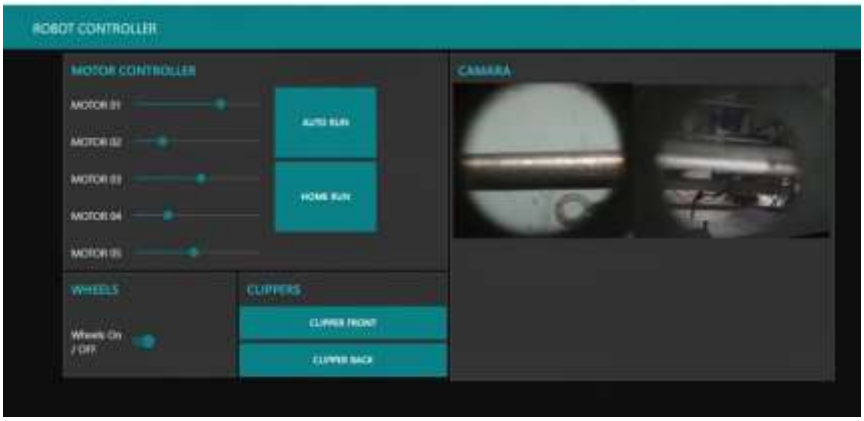


**Figure 9.** Screen output from ground unit while test run

**Conclusion and Recommendations**

This project was based on the Improvement of existing project of "LIVE LINE INSPECTION ROBORT FOR 33 kV TOWER LINES". The main challenge of this improvement was to reduce the overall weight of the system than the previous system. It was a difficult task of using lightweight materials and motors as it has some drawbacks but finally total weight of the robot is reduced by 8 kgs than the previous one. Faraday cage can be used to protect the robot from the possible signal failures from robot to ground unit due to electromagnetic interference caused by the live line.

Even though there are problems faced during construction of prototype, the robot is finally successfully launched. Therefore, it can be suggested that these robots should be introduced to Sri Lankan power system in an effective manner to save the time, cost as well as safety of the labor's life. This robot designed only for inspection purposes of tower lines, but this robot can be improved by adding more capabilities such as repair tower lines using crimping tool, brush the corrosions/fungus and replace corroded pins. This will help to have proper routing maintenance and repair damage without electricity interruptions.

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# OPTICAL CHARACTERISTICS OF CUPRIC OXIDE NANOPARTICLES PREPARED BY HYDROLYSIS OF COPPER ACETATE USING TRIETHYLAMINE

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## Introduction

Metal oxide nanoparticles (MNPs) have received considerable amount of attention in recent past, due to their broad application in various fields of Science and Technology. Because of its natural abundance, low cost production, non-lethal nature with good electrical and optical properties, cupric oxide is mainly used as raw material of different applications of nanoparticles [1].Compared to the p-type semiconductor material with a narrow band gap, copper oxide (band gap 1.2 eV) is useful in various applications such as solar energy conversion ,water splitting reactions, field emission, gas sensors, photovoltaic cells, superconducting materials, thermoelectric materials, sensing materials, glass, ceramics and antimicrobial units [2].

The CuO was prepared by modified alcoholic-TEA method. In the previous study we reported that this synthesized CuO was nanocrystalline p-type CuO with average particle size of 5.7 nm. The flat band potential of the synthesized CuO was 1.04 V. The series resistance and charge transfer resistance of synthesized CuO are lower than those values of commercial CuO as well as bulk capacitance of the synthesized CuO is lower than the commercial CuO [5].In this study, the optical characteristics were analyzed with the current modification of CuO and compared with the commercially available CuO. For further studies, comparison between modified CuO and commercial CuO will be continued on thin film solar cells.

## Materials and Methods

In this study CuO was synthesized using the following method. 0.6 g of copper (II) acetate was added to 60 ml of 96 % methanol at 45-50 $^{o}$ C with vigorous stirring using a magnetic stirrer for 30 min. 4 ml of trimethylamine (TEA) was added to the above reaction mixture and was allowed to continue the reaction at 45-50 °C for 1 hour. A black brown colour CuO precipitate was obtained which was separated out from the reaction mixture and washed with deionized water three times followed by centrifugation at 3600 rpm for 2 min. The washed precipitate was collected into a petri dish using acetone and dried at 150 °C for 30 min. A black brown precipitate of CuO obtained was stored in a desiccator.

The optical characterization of commercial CuO and CuO synthesized in this study were analyzed in the region 400-4000 $cm^{-1}$ using a Fourier Transform Infrared (FTIR) spectrometer (Perkin Elmer Spectrum Two) equipped with an attenuated total reflection (ATR) attachment and UV-Visible spectrophotometer (Genesys 10s UV-Vis).

**Results and Discussion**

The formation CuO was confirmed by the XRD analysis [5]. Figure 1 shows FTIR spectra for commercial and synthesized CuO. Sharp peak at 604 $cm^{-1}$ and 598 $cm^{-1}$ observed in the spectrum for commercial and synthesized CuO respectively are characteristic of Cu-O bond formation. The FTIR spectrum of synthesized CuO shows board absorption band at 3378 $cm^{-1}$ corresponded to the OH stretching while the sharp peak at 2981 $cm^{-1}$ is indicated for C-H stretching [3]. The peak at 1562 $cm^{-1}$ is rather difficult to assign since either the water H-O-H scissoring vibration or the carboxylate anion asymmetrical stretching fall within this range. Sharp peak at 1410 $cm^{-1}$ is attributed to the deformation vibration of the C-H band in $CH_2$ and $CH_3$ groups [4]. Attachment of above groups to synthesize CuO could be expected as the reactants contained all of them.

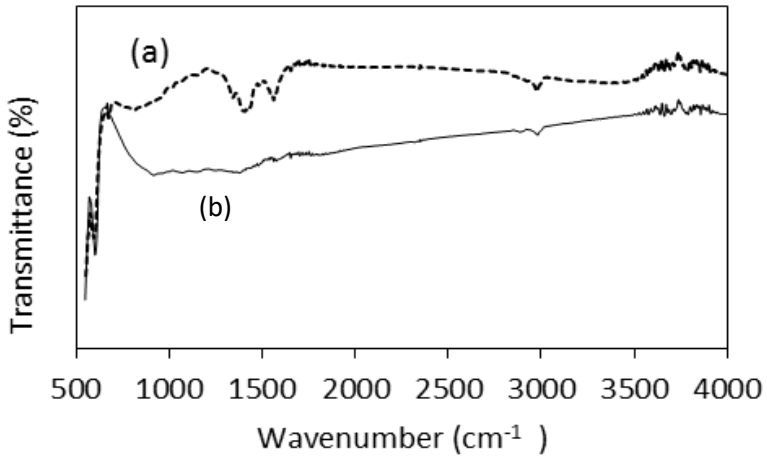


**Figure 1**. FTIR spectrum for (a) Synthesized CuO and (b) Commercial CuO

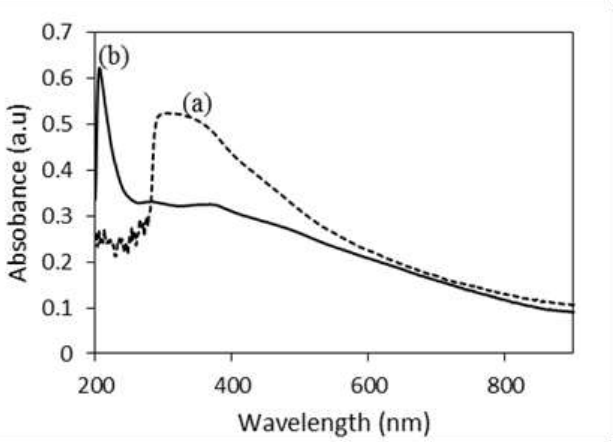


**Figure 2**. UV-visible spectrum for (a) Synthesized CuO and (b) Commercial CuO

Figure 2 shows the absorbance spectra of synthesized and commercial CuO dispersions in methanol. As shown in Figure 2 the synthesized CuO shows maximum absorbance at about 317 nm which is red shifted through a wide range of wavelength from 271 nm to 574 nm. Absorbance spectra of commercial CuO shows sharp peak at 205 nm.

**Conclusion and Recommendation**

Formation of CuO particles were confirmed by XRD analysis. Further analysis of the FTIR spectra showed in addition to Cu-O band, hydroxyl, methyl and carboxylic groups were present in synthesized compound .Comparing the UV-Visible spectra of both type of CuO, the absorption peaks of synthesized CuO is broaden and redshifted which will be advantageous for solar energy conversion and optical devices.

**Acknowledgment**

The authors are grateful for the financial support received from the competitive research grant (GRCS201801) of the Open University of Sri Lanka.

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# TECHNICAL AND ENVIRONMENTAL FEASIBILITY OF CO-FIRING TORREFIED BIOMASS IN A 300 MWe COAL-FIRED POWER PLANT

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## Introduction

Biomass is one of the major renewable sources that can be used as a strategy for the increasing scarcity of fossil fuels. The chemical and physical characteristics of biomass are different to that of coal, but the biomass properties can be upgraded through various treatment processes. In the world, the contribution to the total electricity generation by burning coal is more than 40% and it is 32.4% in Sri Lanka. But coal is considered as the highest $CO_2$ emitting energy source compared to others and recent researches are towards biomass co-firing as a mature solution to reduce $CO_2$ emissions. Additionally, it decreases $SO_x$ and $NO_x$ emissions [1]. Existing coal fired plants can be used with simple modifications for co-firing purposes. However, the lower calorific value, higher hygroscopic nature and moisture content of raw biomass give adverse effects on combustion efficiency and its lower energy density gives the high transportation and logistics cost [1]. Moreover, the fibrous and tenacious nature of the raw biomass affects the inconsistency of particle size and poor grindability. Although researchers showed 20% of biomass co-firing ratio is technically feasible, it is reduced to 5% in real applications [1]. Therefore, raw biomass should be upgraded in order to overcome the above drawbacks, when it is used for industrial co-firing applications.

Torrefaction is one of the thermal pretreatment methods of biomass, which upgrade its thermal, chemical and physical properties and requires 200-300 $^{o}$C temperature, atmospheric pressure under inert environment. During the torrefaction process, oxygen and hydrogen components are partially removed and the resulting solid product has lower Oxygen to Carbon (O/C) and Hydrogen to Carbon (H/C) ratios and higher heating value. The hemicellulose which causes hygroscopicity of biomass are decomposed during torrefaction and a nonpolar unsaturated structure is formed. Thereby the moisture content of torrefied biomass can be reduced to about 3% [2]. Due to breakdown of hemicellulose and depolymerization of cellulose, torrefied biomass becomes brittle and more grindable and it enhances the profile of particle size distribution [1]. Therefore, torrefied biomass can effectively replace raw biomass in co-firing applications.
The Lakvijaya coal power plant at Norochcholai is the main power producer in Sri Lanka which has three generators of 300 MW. Co-firing torrefied biomass with coal is not only environmentally but also economically beneficial for countries like Sri Lanka which have no fossil fuel resources but have numerous

biomass resources. This study was carried out to evaluate the technical feasibility of co-firing torrefied biomass at the Norochcholai coal power plant using Aspen Plus simulation. Gliricidia is an abundantly available energy crop and it is introduced as the fourth plantation crop of Sri Lanka. Therefore, Gliricidia was selected as the biomass source in this study.

## Materials and Methods

*Torrefaction*

Gliricidia was torrefied at 300 $^{o}$C for a residence time of 60 min at atmospheric pressure in an inert environment ($N_2$). The torrefaction was done in a stainless-steel horizontal batch reactor with a nickel chrome heating coil. The reactor was insulated by porcelain accompanied by K-wool. $N_2$ gas was fed continuously throughout the process at a constant flow rate in order to maintain the inert environment and the required temperature.

*Biomass Characterization*

Proximate analysis of torrefied Gliricidia was performed using standard test methods ASTM E871, E872 and E830 for moisture content, volatile matter and ash content respectively and the balance was considered as the fixed carbon content. For the ultimate analysis, solid Perkin Elmer 2400 series II CHN analyzer was used and the remaining was considered as the O content.

The Higher Heating Value (HHV) and Lower Heating Value (LHV) of torrefied Gliricidia were estimated using Equation 1 and Equation 2 respectively [3].

$$HHV = 34.91Y_C + 117.83\,Y_H - 1.51Y_N - 10.34\,Y_O \quad \text{- (1)}$$

$$LHV = HHV - h_v(Y_H + Y_{H_2O}) \quad \text{- (2)}$$

Where,

$Y_X \quad = mass\ fraction\ of\ X; where\ X = C, H, N, O, H_2O$

$h_v \quad = Latent\ heat\ of\ water$

*Process description and Aspen simulation*

The three main co-firing technologies used in the power generation sector are direct co-firing, indirect co-firing and parallel co-firing. In this study direct co-firing method was used as it is the most common and less expensive method for an existing coal fired boiler. The process flow sheet is shown in Figure 1.

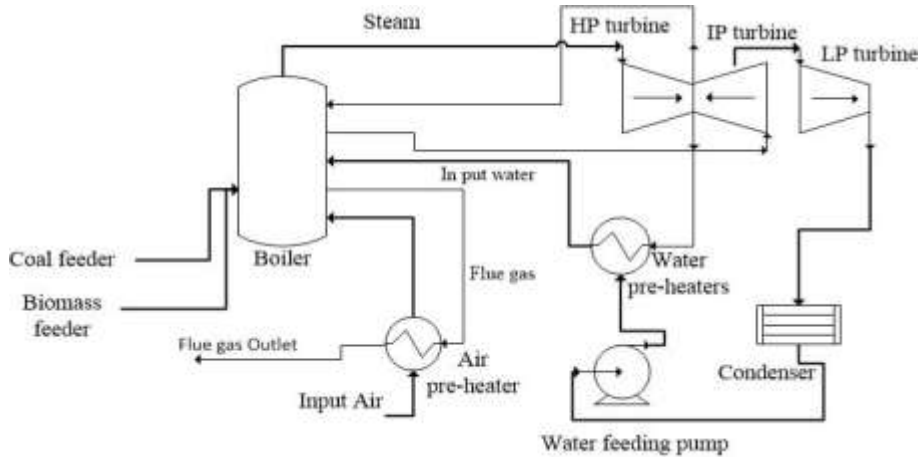


**Figure 1**. Process flow diagram of power plant

The overall process for the coal power plant co-fired with torrefied Gliricidia was simulated using Aspen Plus V10.0. Coal and Gliricidia were defined as non-conventional solids. A combination of a yield reactor and a Gibbs reactor was used to model the combustion chamber in equilibrium. Thermal losses were represented by a cooler connected next to the Gibbs reactor. The boiler is represented using three counter-current heat exchangers. The generated steam is sent through three turbines and a re-heater is used to reheat the steam, which is fed to the next series of turbines. Splitters are used with each turbine to demonstrate the steam extraction for other subunits such as feed water heaters and deaerator. Finally, a condenser followed by a pump is used to represent the continuation of the steam generating cycle. The simulation model which 100% coal case was done by actual data which provide from Norochcholai power plant and 20%, 50%, 75% biomass co-firing cases simulated by maintaining constant thermal input and output. Further $O_2$ in flue gas also maintain at 3.77% in all cases according to actual data.

**Results and Discussion**

Table 1 shows the ultimate analysis, proximate analysis and the LHV of raw and torrefied Gliricidia as well as coal. Since biomass has sulfur content below 0.2%, it can be neglected [4]. The LHV of raw Gliricidia is increased from 13.85 to 17.87 MJ/kg because of the torrefaction process which lowers the O/C and H/C ratios. Therefore, the use of torrified biomass over raw biomass for co-firing is a better option.

**Table 1**. Fuel proximate and ultimate analysis of fuels

| | Ultimate analysis (dry %) | | | | | Proximate analysis (wet %) | | | | LHV (MJ/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| | **C** | **H** | **O** | **N** | **S** | **V.M** | **F.C** | **Moisture** | **ash** | |
| Raw Gliricidia | 42.13 | 6.72 | 46.23 | 0.84 | n/a | 60.10 | 20.84 | 15.63 | 3.44 | 13.85 |
| Torrefied Gliricidia | 45.59 | 6.33 | 42.49 | 0.73 | n/a | 66.20 | 26.09 | 3.00 | 4.71 | 17.87 |
| Coal | 66.57 | 4.40 | 13.30 | 1.20 | 0.6 | 21.8 | 57.9 | 7.4 | 12.9 | 27.79 |

**Table2**. Total fuel flow rates and $CO_2$ emissions in different co-firing ratios

| Co-firing ratio | **0%** | **20%** | **50%** | **75%** |
|---|---|---|---|---|
| Coal (ton/h) | 114 | 91.31 | 57.06 | 28.53 |
| Torrefied biomass (ton/h) | 0 | 32.52 | 81.31 | 121.95 |
| Total flow (ton/h) | 114 | 123.83 | 138.37 | 150.48 |
| Output power generation (MW) | 296.4 | 296.4 | 296.4 | 296.4 |
| Net $CO_2$ emission (kg/MWh) | 844.49 | 777.96 | 484.05 | 238.20 |

The required fuel flow rates and resulting net $CO_2$ emissions are summarized in Table 2 for different co-firing ratios. Since biomass is $CO_2$ neutral, its combustion does not contribute net $CO_2$ emission and when increasing co-firing ratio, net $CO_2$ emission is reduced. The figure 2 represents the $SO_X$, $NO_X$ and CO emissions related to different co-firing ratios. It shows the amount of $SO_X$, $NO_X$ and CO emissions are significantly reduced with increasing co-firing ratios. The lower nitrogen and sulfur content in biomass are the main reason for low $NO_X$ and $SO_X$ emissions. Literatures also show similar results for co firing biomass with coal [5]. Moreover, the higher volatile matter content of biomass than coal, initiates predominant combustion at the gas-phase and reduces $NO_X$ emission during the biomass combustion [5]. An experimental investigation on co firing also has shown lower $SO_X$, $NO_X$ and CO emissions with the biomass co-firing. Further, they have suggested that the co-firing with torrefied biomass improves the combustion efficiency and results in lower CO concentrations.

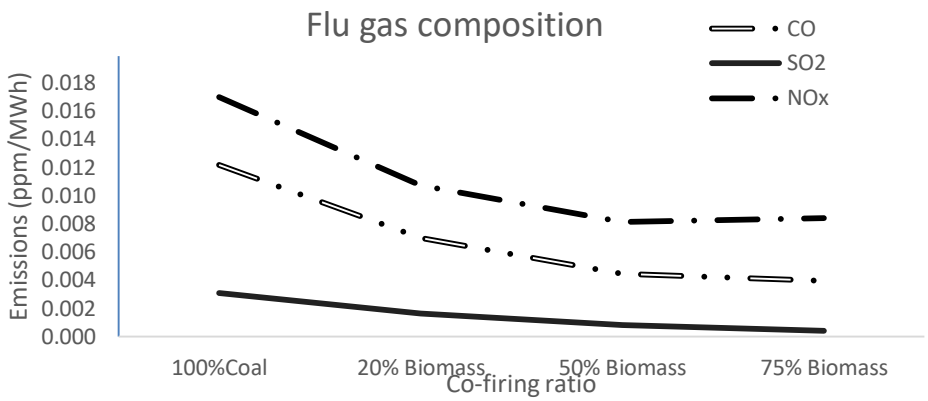


**Figure 2**. Emissions in different co-firing ratios

## Conclusions and Recommendations

From the results obtained by the Aspen Plus simulation, it can be seen that the temperature of the flue gas remains at a high value, it can be further used for the Gliricidia torrefaction process. Therefore, the cost of biomass torrefaction could be covered within the process itself. The $SO_X$ and $NO_X$ emissions have lowered with the increase of biomass cofiring percentage. Thereby the environmental concerns are reduced to a greater extent. The amount of net $CO_2$ emitted by the process is inversely proportional to the co-firing percentage due to carbon neutrality of biomass. Though the co-firing biomass percentage can be 75% or even higher, the combustion chamber capacity is a crucial factor. Since this research is done for an existing coal power plants those limitations should be considered.

## Acknowledgements

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# FOCUS AREA

## Environment

# HABITAT-COVER ASSESSMENT IN THE KUMANA NATIONAL PARK, SRI LANKA USING MULTI TEMPORAL SATELLITE DATA

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## Introduction

National parks of Sri Lanka are a rich tapestry of different habitats, each supporting a unique assemblage of plants and animals. However, relatively few pristine landscapes exist today. Various natural or anthropogenic factors [1] have directly or indirectly impacted habitats causing changes at temporal and spatial scales [2], influencing their potential to sustain biota. Thus, information on such changes would be crucial for effective management of natural landscapes. Remote sensing (RS) integrated with Geographic Information System (GIS) and Global Positioning System (GPS) is one of the effective techniques that can generate accurate spatial information about temporal and spatial changes in land cover and habitat characteristics [3].

Kumana (Yala-East) National Park (NP) ($6^0$ 30' to $6^0$ 42' N, $81^0$ 04' to $81^0$ 15' E), which lies on the south-eastern coast of Sri Lanka, is an important habitat for many species of flora and fauna, particularly for its large mammal and avifaunal communities. Recent observations have revealed that grassland habitats have reduced in extent whist the nesting birds have also declined [4]. The present study, therefore, attempted to assess habitat cover change within the Kumana NP which occurred over the past 15 years using available satellite images. The specific objectives were to identify the diverse habitat types, generate a sequence of habitat cover maps and quantify changes in habitat cover.

## Materials and Methods

The study was carried out for the years 2004, 2005, 2009 and 2019 using the available satellite maps from Landsat 5 Thematic Mapper (TM) and Landsat 8 Operational Land Imager (OLI) (http://www.usgs.gov). Five major habitats namely forest, grasslands, water body, bare land and coastal vegetation were identified. Satellite images with least cloud coverage were selected and these were classified in ArcGIS version 10.7 using the unsupervised classification method. Different habitat covers were identified based on ground truthing and high-resolution images from Google Earth. Comparisons were limited by the unavailability of satellite images for the same months over the different years.

**Results and Discussion**

Visual interpretations of the images (Figure 1) revealed temporal changes in the different habitat types over the past 15 years. The most notable changes were in the forest and grassland habitats (Figure 2). The forests declined sharply from 2004 to 2005 by 14.36 % (from 129.44 $km^2$ to 101.52 $km^2$), but steadily increased thereafter by 11.8 % (from 101.52 $km^2$ in 2005 to 124.43 $km^2$ in 2019). The grasslands on the other hand showed a sharp increase over 2004 to 2005 by 9.73% (from 53.39 $km^2$ to 71.87 $km^2$) but has declined thereafter by 7.43 % (from 71.87 $km^2$ in 2005 to 57.80 $km^2$ in 2019). The temporary changes in forest and grassland habitats in 2005, though in opposite directions, were most likely due to the impact of the tsunami. It appears that the destruction of forest habitats facilitated the increase in grasslands. The forest cover in 2019 is still lower than that at the pre-tsunami stage.

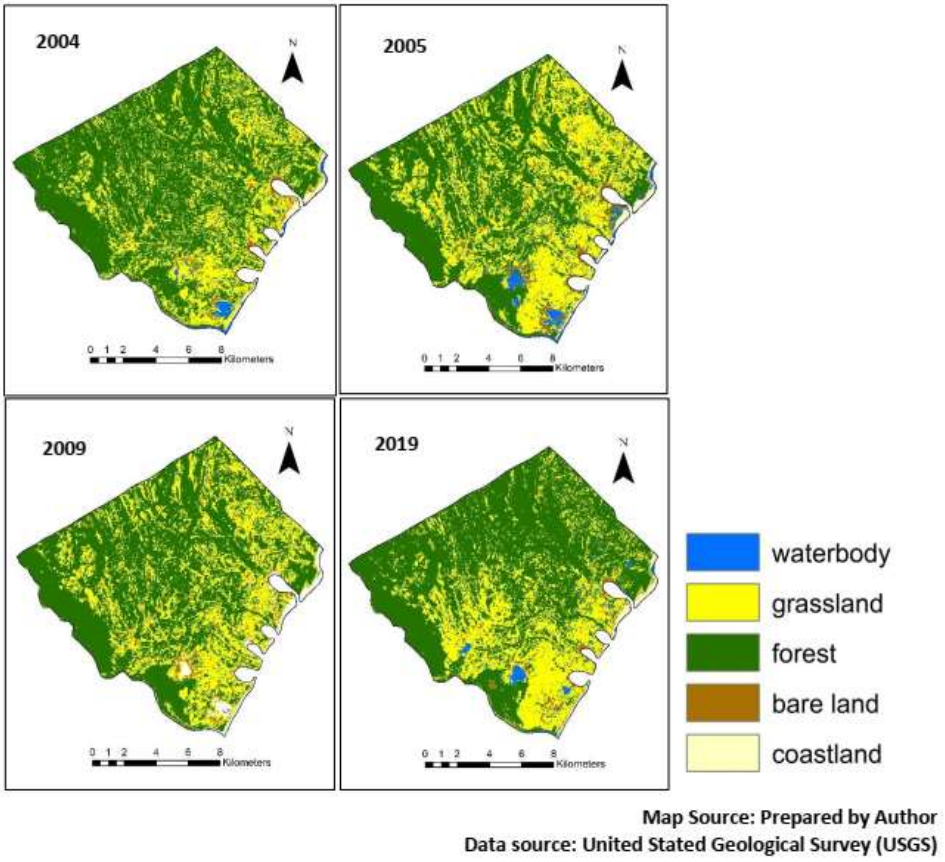


**Figure 1.** Habitat cover maps of the Kumana NP for the years 2004, 2005, 2009 and 2019

The post tsunami changes in forest and grassland habitats are most likely due to direct or indirect anthropogenic factors such as the release of domestic cattle,

spread of invasive species such as *Lantana camara, Opuntia dillenii* and *Chromolaena odorata* and Chena cultivation and burning of grasslands around its periphery.

The increase in forest cover was more pronounced along the border of the Kumana NP which is contiguous with the Yala Strict Natural Reserve, which is likely due to the greater protection received. No significant changes were noted in the extents of bare lands or coastal vegetation which include mangroves. Water bodies show a slight increment in area in the past 10 years probably due to the creation/expansion of waterholes as a management activity. No habitat type has been completely lost during the study period.

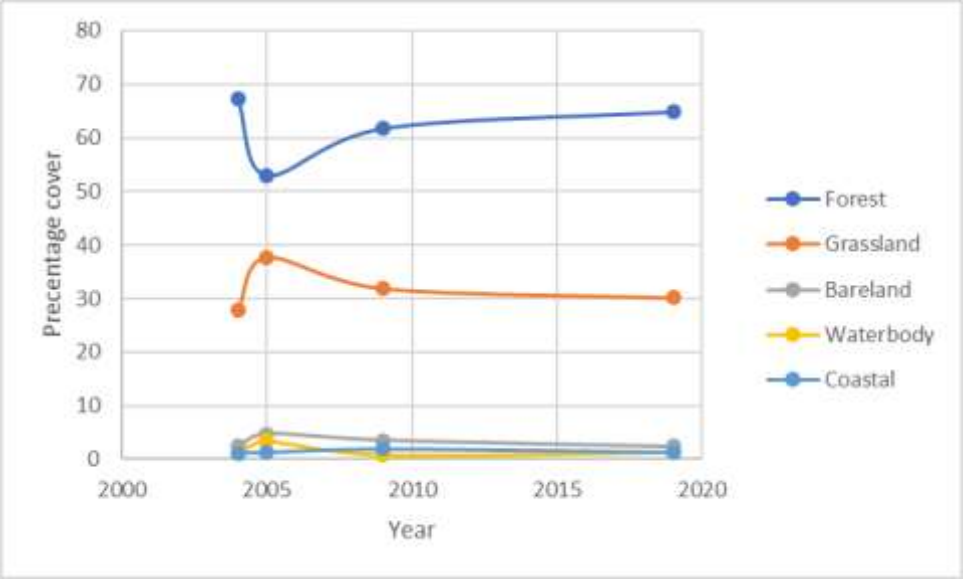


**Figure 2.** Temporal changes of habitats in the Kumana NP from 2004 to 2019

**Conclusions and Recommendations**

This study demonstrates that multi temporal satellite data-based maps can effectively assess habitat cover changes in landscapes over space and time. The most notable changes in cover in the Kumana NP over the past 15 years were in forest and grassland habitats. The study clearly demonstrates the effect of the tsunami on habitat cover, while the post tsunami changes were most likely due to direct or indirect human influences. These findings can be made use of to devise suitable restorative strategies that would ensure the maintenance of habitat proportions at an optimal level. Furthermore, these results would serve as a baseline for future management and conservation of this important wildlife habitat.

**Acknowledgement**
Finances assistance from the University of Colombo is acknowledged.

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# ASSESSMENT OF PHYTOPLANKTON COMMUNITY STRUCTURE AND SPECIES DIVERSITY IN DIYAWANNAWA WETLAND

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## Introduction

Wetland ecosystems are home for diverse groups of plant and animal communities. Among them, phytoplankton can play a major role as an important component in wetland food webs. Further, phytoplankton assemblages are used in wetland water quality monitoring programs. Diyawannawa wetland system is a major wetland that contributes to flood management in the commercial capital of Sri Lanka. In addition, this wetland is home for high avian and plant biodiversity. However, in some parts of the wetland, frequent occurrence of algal blooms is observed in the recent past. Under certain environmental conditions, tropical wetlands can experience phytoplankton or algae blooms resulted due to rapid rate of reproduction and quick multiplication within a short time. Therefore, it is important to frequently assess the diversity and distribution of phytoplankton assemblages in different parts of the wetland in order to predict the possibility of occurring phytoplankton and algal blooms. The objectives of the present study was to assess the variation and community composition of phytoplankton assemblages at selected sites of the Diyawannawa wetland system in order to identify the dominant phytoplankton species that can possibly result in the occurrence of algal blooms.

## Materials and methods

Seven study sites associated with input streams to the Diyawannawa wetland were selected. Sites A (6° 54.371'N 79° 54.533'E), B (6° 54.379'N 79° 54.199'E), C (6° 54.234'N 79° 54.535'E) and G (6° 54.590'N 79° 54.264'E) were located in the northern portion of the wetland and sites D (6° 53.500'N 79° 54.642'E) , E(6° 53.493'N 79° 55.228'E) and F (6° 52.658'N 79° 55.612'E) were located in the southern portion. At each site, three replicate phytoplankton samples were collected using the phytoplankton net (50µm mesh size) at 0-50 cm depth and they were immediately preserved in Lugol's solution and transported to the laboratory. In the laboratory, they were identified to the lowest possible taxonomic level using relevant standard taxonomic keys. Sampling was conducted in May, July, August and September 2018 to cover both rainy and dry seasons. Phytoplankton abundance data were used to calculate species richness, diversity and evenness indices. Species richness was determined by

Margalef index [1] and Menhinick index [2]. Shannon wiener index [3] and Simpson's index [4] were used to determine species diversity and species evenness was determined by Shannon's equitability. Individual rarefaction diagrams for different classes of phytoplankton were developed. Data analysis was conducted using PAST 3.0 software package.

**Results and Discussion**

A total of 50 species of phytoplankton were identified belonging to five classes: Class Cyanophyceae: 13 species; Class Chlorophyceae: 18 species; Class Zygnematophycae: 10 species; Class Bacillariophyceae: 6 species; Class Euglenophyceae: 3 species. Highest mean abundance of phytoplankton was recorded in site D (11882 /L) which had domestic waste inputs and lowest mean abundance was in Site F (2592 /L). The species richness, diversity and evenness indices calculated for each site is given in Table 1.

**Table 1**. Phytoplankton species richness, diversity and evenness indices calculated for selected sites in the Diyawannawa wetland system in 2018.

| | Sites | | | | | | |
|---|---|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** | **F** | **G** |
| Margalef index | 4.39 | 4.36 | 4.40 | 4.02 | 4.16 | 4.95 | 4.52 |
| Menhinick index | 0.51 | 0.42 | 0.52 | 0.31 | 0.54 | 0.78 | 0.88 |
| Shannon wiener index, | 2.04 | 1.72 | 2.03 | 1.76 | 1.54 | 1.80 | 2.63 |
| Simpson's index | 0.77 | 0.68 | 0.76 | 0.71 | 0.56 | 0.66 | 0.87 |
| Shannon's equitability | 0.56 | 0.46 | 0.56 | 0.48 | 0.43 | 0.49 | 0.75 |

Both the Menhinick's and Margalef's indices measure species richness in an ecosystem. In the present study, Menhinick index ranges from 0.31 to 0.88 and Margalef's index ranges from 4.02 to 4.95. Both the indices indicate lowest species richness at site D. However, according to Margalef's index, highest species richness is in Site F and Menhinick index records highest richness in Site G (Table 1).

The Simpsons index considers both the number of species present and well as the abundance of species as a measure of biodiversity. Shannon Weiner index (H´) takes into account species richness and spices evenness components as overall index of diversity. The higher the values of Simpson's index and H´, greater the species diversity. Shannon wiener index of the present study ranges from 1.54 to 2.63 and the Simpsons index ranges from 0.56 to 0.87. Both the diversity indices indicate highest phytoplankton diversity at site G and lowest at site E.

Further, Shannon Weiner index (H´) of phytoplankton can be used to predict the water quality of the ecosystem based on a categorical classification: H´ > 3:

clean water; 1≤ H´≥3: moderately polluted water; H´<1: heavily polluted water [7]. In the present study the H´ indicates a moderately polluted status in all the study sites.

The Shannon's equitability of the study sites ranged from 0.43 to 0.75 indicating high species eveness in Site G compared to other sites. The individual rarefaction diagrams of class Chlorophyceae, Cyanophyceae and Zygnematophyceae showed similar patterns. The individual rarefaction diagrams of class Chlorophyceae is given in Figure 1 as a representation of classes Cyanophyceae and Zygnematophyceae as well. Highest number of Chlorophyceans, Cyanophyceans and Zygnematophyceans were recorded from Site D. However, all the other sites contain more number of species with less representatives from each species. Therefore, individual rarefaction curve of Chlorophyceans, Cyanophyceans and Zygnematophyceans indicate higher species homogeneity in site D and higher evenness in other sites.

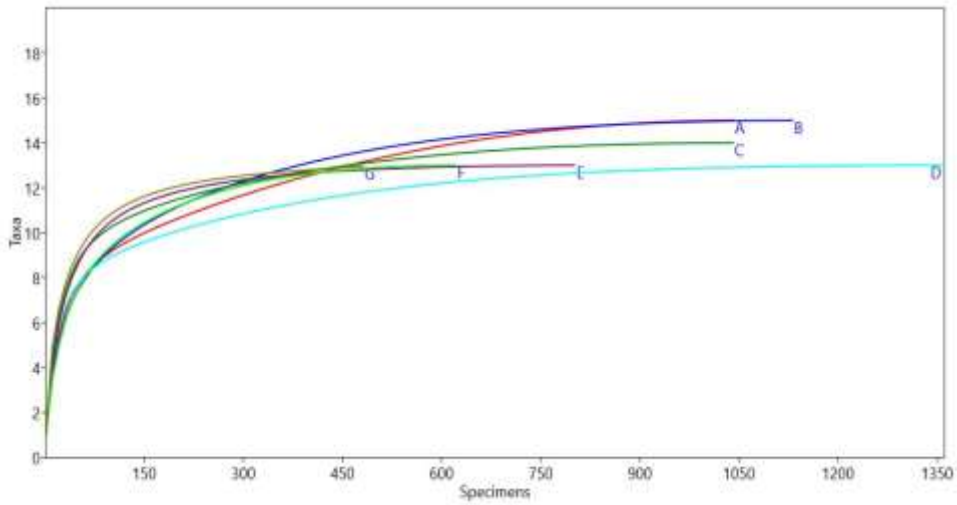


**Figure 1.** The individual rarefaction diagram of cyanophyceans in the selected sites of the Diyawannawa wetland in 2018

The individual rarefaction diagram of class Euglenophyceae is given in Figure 2. Highest number of Euglenophyceans are recorded from Site D. S ites B, C, E and F contain more number of Euglenophyceae species with less representatives from each species. Therefore, individual rarefaction curve of Euglenophyceans indicate higher species homogeneity in site D and higher evenness in sites B, C, E and F. Sites A and G had less diversity and less evenness of Euglenophyceans.

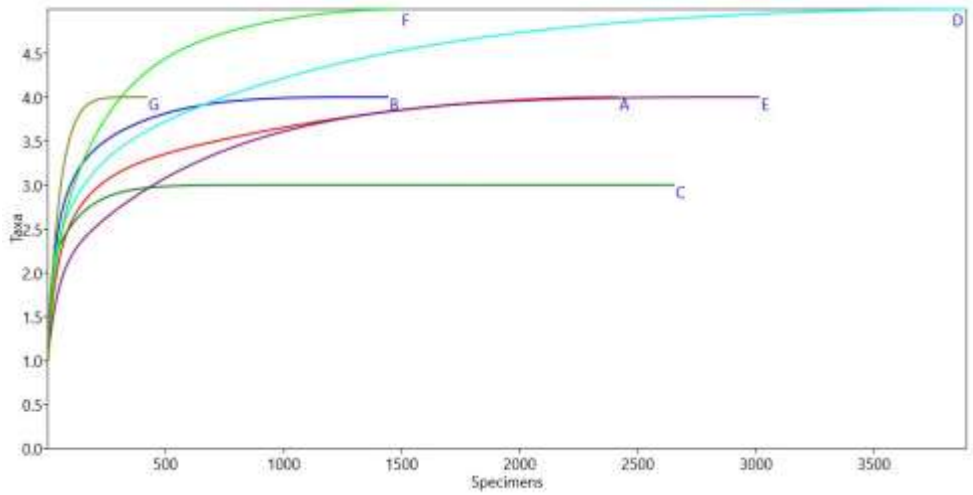


**Figure 2.** The individual rarefaction diagram of Euglenophyceans in the selected sites of the Diyawannawa wetland in 2018

The individual rarefaction diagram of class Bacillariophyceae is given in Figure 3. Highest number of Bacillariophyceans are also recorded from Site D. However, the maximum evenness of Bacillariophyceans is also recorded from Site D. In addition, sites B, C E and F shows higher evenness and Sites A and G shows highly homogeneous distribution of Bacillariophyceans.

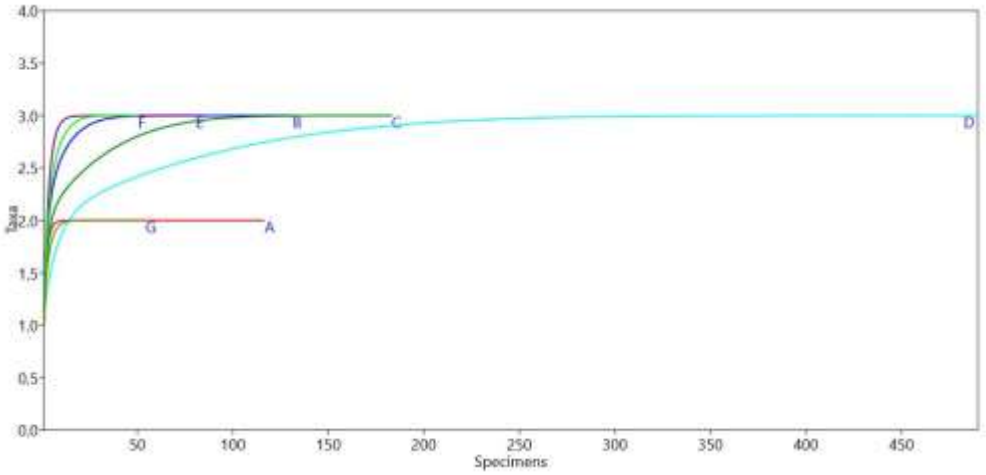


**Figure 3.** The individual rarefaction diagram of Bacillariophyceans in the selected sites of the Diyawannawa wetland in 2018

**Conclusions and Recommendations**

The results of the present study identifies Site D as the site with highest number of phytoplankton belonging to each class. However, as the species evenness in this site is comparatively low, several dominant species of phytoplankton of each class (*Spirulina* sp, *Pediastrum simplex*, *Closterium kuetzingii*, *Melosira* sp, and *Euglena* sp from Classes Cyanophyceae, Chlorophceae, Zygnematophyceae, Bacillariophyceae and Euglenophyceae respectively) seems to be dominating and outcompeting other species. Therefore, it is recommended to continuously monitor these phytoplankton assemblages in order to identify these dominant phytoplankton and to manage their excessive multiplication. Further, it is recommended to continuously monitor water quality and identify the seasonal and spatial trends of water quality variations which can be helpful in wetland management.

Although the number of individuals recorded from other sites are comparatively low, the evenness of phytoplankton in most sites are high, indicating higher diversity. Class Cyanophyceae, which is considered as a major group responsible for algal blooms also shows high evenness in almost all the sites. Therefore, it is very important to continuously monitor the abundance and distribution of these organisms in order to control the sudden occurrence of algal blooms.

In addition, other classes of phytoplankton recorded in the present study, were also showing uneven distribution with highest abundance in Site D. Therefore, it is recommended to identify possible sources of nutrients to this region of the wetland and to conduct suitable nutrient management programs and to continuously monitor phytoplankton diversity and nutrient input as a management action to control the environmental conditions that can possibly result in phytoplankton blooms in the Diyawanna Wetland system.

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# CHROMOSOMAL ABNORMALITIES INDUCED BY THE TREATED TEXTILE EFFLUENTS

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## Introduction

Discharge of treated and untreated textile effluents into the aquatic environment can have detrimental effects to the biological communities residing in those environments. Various toxic chemicals are used for textile manufacturing process and these chemical are discharged as wastewater. Textile effluent treatment process can neutralize most of these toxic chemicals. However, these treated textile effluents can be contaminated with genotoxic chemicals even in trace amounts. The *Allium cepa* test is a widely used plant bioassay to assess the genotoxicity through the analysis of chromosomal aberrations. The objective of the study was to assess the chromosomal abnormalities in the *Allium cepa* root tip cells exposed to water from a canal of Kelani River receiving treated textile effluent.

## Materials and Methods

The study was conducted associated with a discharge canal of a textile manufacturing industry located near Kelani river. A tributary that received treated textile effluent from textile industry was selected as sampling location. Four sampling sites (A to D) were selected along the tributary (A: effluent discharge point, B: 100m downstream from site A along the tributary; C: 200m downstream from site A along the tributary; D: 100m upstream from site A along the tributary). Wastewater samples were collected from each site for the toxicity analysis. Aged aerated tap water was used as a control. Sampling was carried out in three occasions from June to September in 2018 covering both dry and rainy seasons. The *Allium cepa* bioassay was conducted as described by Grant (1982). 10 0nions bulbs were placed on each site and aged tap water. The descriptive statistics in the MINITAB 14.0 version were used to obtain the mean and the standard error of the mean and One-way ANOVA was used to assess the spatial variation of chromosomal aberrations.

## Results and Discussion

The observed chromosomal abnormalities are presented in Figure 1. C-metaphase, chromosomal adherence, bridges, disturbed anaphase, vagrant chromosomes and chromosomal breaks were the observed chromosomal abnormalities during the study (Figure 1). The mean total chromosomal

abnormalities observed at each sampling event are presented in Table 1. Total chromosomal abnormalities in discharge point (Site A), 100m downstream point (Site B), 200m downstream point (Site C) and 100m upstream point (Site D) were significantly higher than that of the aged tap water in all sampling events. Chromosomal abnormalities were significantly high at discharge point and less at 100m upstream point. The most frequent abnormalities were chromosomal adherence and vagrant chromosomes. Chromosomal adherence may occur due to increased chromosomal contraction and condensation. Presence of chromosomal adherence is considered as a common sign of toxic effects of chemicals on chromosomes and probably leading to cell death [1]. The unequal distribution of chromosomes or paired chromatids leads to the occurrence of vagrant [2]. C-metaphase and bridges were also recorded in a considerable amount. Stickiness of chromosomes prevent complete separation during anaphase and it may lead to a rise of chromosomal bridges [3]. Chromosomal bridges and breaks are categorized as an indicator of a clastogenic effects which leads to alteration in DNA structure. Chromosomal abnormalities associated with aneugenic effects are chromosome losses, delays, adherence, multipolarity and C metaphase [4]. Chromosomal breaks were the most uncommon abnormality. The occurrence of these type of abnormalities in the chromosomes of *Allium cepa* indicate the presence of genotoxic agents in the textile effluents [5]. According to the results, discharge point had the highest number of chromosomal abnormalities and this suggests that it is the most toxic of the sampling sites. This may be due to the presence of heavy metals and other mutagenic substances in the selected textile effluent. However, there was a decreasing trend of abnormalities when going downstream from the discharge point.

**Table 1**. Total chromosomal abnormalities in root meristematic cells of *Allium cepa* exposed to wastewater samples collected from different sites and aged tap water in the three sampling events.

| Site | Total chromosomal abnormality (‰) | | |
|---|---|---|---|
| | June | August | October |
| Site A | $250.0 \pm 20.0^{a}$ | $287.9 \pm 12.4^{a}$ | $246.2 \pm 17.9^{a}$ |
| Site B | $208.3 \pm 21.6^{ab}$ | $233.1 \pm 18.8^{ab}$ | $191.7 \pm 10.5^{ab}$ |
| Site C | $180.5 \pm 10.9^{b}$ | $196.0 \pm 17.9^{bc}$ | $175.0 \pm 11.2^{b}$ |
| Site D | $106.0 \pm 9.0^{c}$ | $144.9 \pm 11.2^{c}$ | $113.6 \pm 10.8^{c}$ |
| Aged tap water | $66.3 \pm 4.1^{d}$ | $48.6 \pm 4.3^{d}$ | $68.9 \pm 10.8^{d}$ |

Data are presented as mean ± SEM. Means values indicated by different superscript letters in each column are significantly different from each other (ANOVA, Tukey's test,$p<0.05$). Site A: discharge point, site B: 100m downstream point, site C: 200m downstream point and site D: 100m upstream point

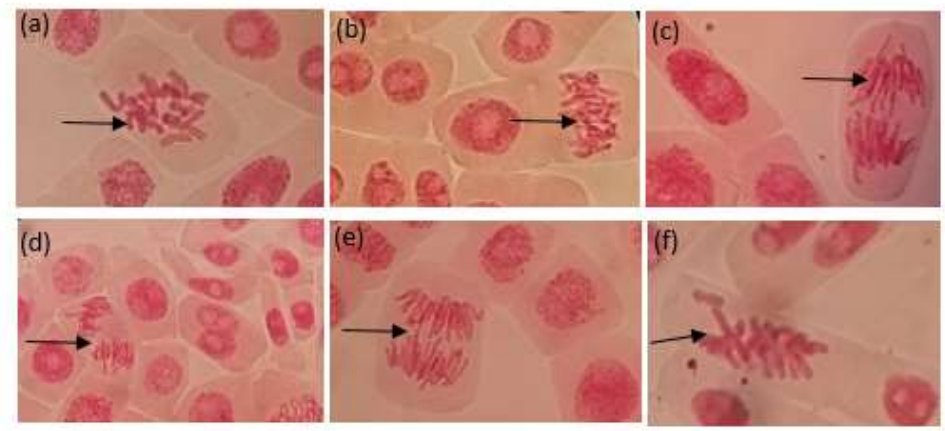


**Figure 1.** Chromosomal abnormalities seen in *Allium cepa* root meristem following 48 hour exposure to different wastewater samples and aged tap water. (a)-C-metaphase, (b)-chromosomal adherence, (c)-vagrant, (d)-disturbed anaphase, (e)-chromosomal bridge, (f)-chromosomal break.

## Conclusion

The results of this study indicated that the treated textile effluents that are directly discharged into the canal of Kelani River can contain compounds that can affect at the chromosomal level of exposed organisms. Presence of these compounds even in trace amounts can cause direct and indirect toxic and detrimental effects to environment as well as to human and animal health. Therefore, it is recommended that the wastewater be properly treated for trace amounts of chromosome affecting compounds before being discharged into the canal.

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# ASSOCIATION BETWEEN THE ABUNDANCE OF BUFFALOES AND NUTRIENT LEVELS IN WATER HOLES IN THE KUMANA NATIONAL PARK, SRI LANKA

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## Introduction

Kumana National Park, which is contiguous with the Yala National Park is located in the Eastern Province of Sri Lanka. The park is renowned for its avifaunal community, probably due to the presence of extensive marshes and villu habitats. The park also supports many charismatic mammals such as the elephant, sloth bear, leopard and several ungulates. As highlighted in the Kumana NP Management Plan [1], it is thought that the presence of buffaloes is detrimental to the long-term survival of other herbivores in the park.

The park is reported to support two species of buffaloes (Family Bovidae), the swamp water buffalo (*Bubalus bubalis)* and the Asiatic wild water buffalo (*B. arnee*), the later a native which is classified as an Endangered species in Sri Lanka according to the IUCN classification and the former being an introduced species. The two species co-occur within the park and are suspected to interbreed, posing a threat to the survival of the native buffalo [1]. Excessive grazing pressure and degradation of waterholes by the buffaloes have been highlighted as possible causes for concern for the survival of other mammalian species.

The present study was carried out in the Kumana National Park to identify associations between the abundance of buffaloes and selected chemical properties of waterholes utilized by these buffaloes. The study also aimed to assess buffalo abundance, characterize herds based on species, age composition, record their behaviour and document habitat use.

## Materials and methods

The study was conducted at the Kumana National Park in November 2019 which falls within the wet season. Assessment of buffaloes were conducted along a 35.5 km stretch of the road network between 0700 and 1200 h. Vehicle counts were conducted along the selected stretch, which traversed through grasslands, scrublands, villus, water holes, lagoons and mangrove swamps (See Figure 1). All buffaloes sighted were counted along with the type of habitat in which they were observed. Additionally, six water holes were randomly selected

(georeferenced) and separate counts were taken. At each of these sites, buffalo herds (if present) were characterized based on morphology and age classes (adults and young). Each buffalo was photographed to examine for typical *B. arnee* or *B. bubalis* characters which were body colour, orientation of the horns, colour of hair tuft between the horns and colour of the tail bristles [2].

Behaviour was recorded using scan sampling in relation to the categories: grazing/feeding, defecating, wallowing, resting and courtship (n= 6 herds; for 30 minutes at 10 minute intervals). Five surface water samples (below a depth of 3 cm) were collected from each water hole to test for phosphate and nitrate levels using the spectrophotometric methods – Phospho-vanadomolybdate and sodium salicylate methods, respectively [3].

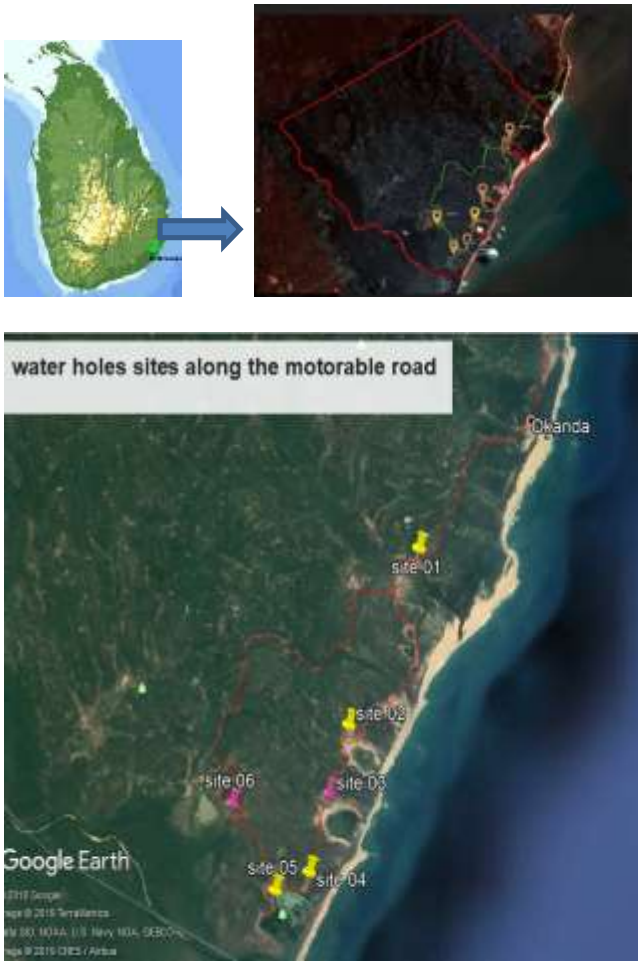


**Figure 1.** The Kumana National Park and the water holes where observations on buffaloes were conducted.

**Results and Discussion**

A total of 99 buffalos were recorded along the 35.5 km of trail and of these approximately 89 % were recorded in close proximity to water holes, while the other 11% were recorded in forest habitat. The mean (± SD) number of buffalos per water hole was 7 (46 maximum -3 minimum). Of the buffalos observed, 77 % were classified as adults, while the rest were calves [4]. The calves were always in the company of the adults.

Based on morphological features, both *B. arnee* and *B. bubalis* morphotypes were present. At all water holes, the buffaloes that exhibited characteristics of the introduced species (*B. bubalis*) was greater than the numbers having traits of the wild buffaloes (*B. arnee*) (Table 1). Calves were not characterized in terms of species due to the inability to differentiate them using the documented traits [4].

**Table 1.** Composition of buffalo herds at six water holes in the Kuman National Park in the wet season

| Water hole | Buffalo count | Adults | Juveniles | Suspected wild buffaloes | Suspected feral buffaloes |
|---|---|---|---|---|---|
| 1 | 46 | 38 | 8 | 13 | 25 |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 3 | 9 | 7 | 2 | 2 | 5 |
| 4 | 3 | 3 | - | 3 | - |
| 5 | 30 | 20 | 10 | 3 | 17 |
| 6 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **88** | **68** | **20** | **21** | **47** |

Table 2 reports findings in relation to the behavioral observations. Overall 47 % of the buffaloes were wallowing when observed whilst 40% were engaged in feeding, 12 % resting and 1% defecating. Reports from elsewhere have indicated that buffaloes could cause considerable damage to the environment through these activities. As observed in this study wallowing was a predominant behavior displayed by the buffaloes. This action stirs up the mud making the water holes unsuitable for many aquatic fauna and flora as well as other terrestrial ungulates and the elephants [5]. Many were also seen to feed on grass on the banks of the water holes which they shared with deer, sambhur, wild boar and elephants. Only two elephants were observed in the vicinity of the water holes. However, excessive populations of buffaloes may cause adverse impacts for other herbivores sharing the same habitat, particularly during the dry season when food is scarce. Interestingly other herbivores were not observed at water holes that were dominated by buffalos.

**Table 2.** Frequency of behavioral types of buffalos in relation to the site observed.

| Water Hole | Abundance | Behavior type | | | | |
|---|---|---|---|---|---|---|
| | | Feeding | Defecating | Wallowing | Courtship | Resting |
| 1 | 46 | 11 | 1 | 28 | 1 | 5 |
| 2 | 9 | 6 | 0 | 3 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 3 | 2 | 0 | 1 | 0 | 0 |
| 5 | 30 | 16 | 0 | 9 | 0 | 5 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **88** | **35** | **1** | **41** | **1** | **10** |

The majority of the buffalos were observed at the periphery of the water holes with only a few at the center. Defecation and urination were not frequently observed, although many dung piles were observed at the periphery of the tanks. The phosphate concentrations in the water holes varied from 0.055ppm to 0.744 ppm, while the respective levels for nitrates were 1.388 ppm to 8.414 ppm (See Table 3). There was a highly significant positive correlation between buffalo abundance and the phosphate levels (Pearson's correlation r = 0.95, P<0.05) and the nitrate levels (Pearson's correlation r = 0.93, P<0.05).

**Table 3.** Phosphate and nitrate levels (Mean ± Std. deviation; n = 5 per water hole) in relation to buffalo counts.

| Water hole | Buffalo count | Phosphate concentration (ppm) | Nitrate concentration (ppm) |
|---|---|---|---|
| 1 | 46 | 0.744±0.126 | 8.414±1.197 |
| 2 | 0 | 0.055±0.044 | 1.388±0.274 |
| 3 | 9 | 0.362±0.009 | 2.149±0.251 |
| 4 | 3 | 0.165+0.008 | 1.859±0.287 |
| 5 | 30 | 0.446+0.005 | 3.526±0.274 |

**Conclusions and Recommendations**

The present study has shown that there is a relatively high abundance of buffaloes at water holes in the Kumana National Park (in comparison to forest habitats) the majority of them bearing the characteristics of feral buffaloes (*B. bubalis*). Grazing by the buffaloes may trigger competition with other ungulates such as the deer and sambhur. The positive association between the abundance of buffaloes at water holes and nitrate and phosphate levels of those water holes, suggests that activities such as wallowing and defecation which have the potential to cause nutrient enrichment could adversely impact other faunal species sharing the same waterholes.

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# EVALUATION OF HEAVY METAL DISSOLUTION MECHANISMS FOR BATTERY INDUSTRIAL SLUDGE

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## Introduction

Generation of waste material as by-products from industrial processes became a worldwide concern when considering the cost associated with waste management. Amongst various kind of industrial waste products, wastewater sludge has been considered as a waste product that requires extensive treatment as they tend to contain a high amount of toxic heavy metals. Release of those toxic metals into the environment caused a wide range of environmental consequences as well as human health-related problems [1]. In Sri Lanka, sludge generated through battery industry is discharged to the environment either directly or after treating partially due to insufficient facilities used in industrial wastewater treatment plants [2]. Previous studies revealed that the battery industrial sludge contained high concentrations of heavy metals including, Pb, Ni, Cd, Ni, Zn, and Fe [3].

Rapidly developing industrial activities throughout the world caused for a high demand for metals as raw materials for various products. Therefore, recovering metals from waste products became more appealing mainly due to the depletion of natural ores with high metal consumption. With that regard, acid leaching has been successfully incorporated for recovering metal from different kinds of industrial wastes. That technique frequently utilizes different types of inorganic acids such as sulfuric, nitric, hydrochloric and phosphoric as well as a few organic acids including malic, acetic, citric and succinic acids. These acids have been studied for their effectiveness as leaching solvents on the recovery of metals from wastes [4]. However, none of the studies access the effectiveness of acid leaching technique for recovery of heavy metals from battery industrial sludge as it provides a good source for metals. Furthermore, understanding of metal releasing mechanisms from industrial sludge with acid leaching can be considered as an important aspect as different acid types have shown different leaching performance for metals from industrial wastes. Therefore, the objective of this study was to access the effect of the type of extractant, concentrations of the extractants, and solution pH in order to investigate ligand and proton effect on heavy metal extraction.

**Materials and Methods**

The sludge that collected from a wastewater treatment plant of a battery manufacturing industry was air-dried, mechanically crushed, and sieved with one-millimeter mesh and the fraction < 1 mm was used for characterization and leaching experiments with acids. Chemical parameters, including pH, electrical conductivity (EC), available nitrogen and phosphorus, percentage of total organic carbon (TOC), and cation exchange capacity (CEC) of sludge were analyzed using the standard procedures presented in Anderson and Ingram [5]. The chemical composition of battery industrial sludge was determined by the oxide mode of X-ray fluorescence spectrometer (XRF, Shimadzu-1800), operated at 40 kV, 95 mA. Leaching experiments were done with three inorganic acids (*i.e.*, nitric, sulfuric, and phosphoric) and three organic acids (*i.e.*, acetic, malic, and citric) at concentrations ranging from 0.1 to 2.0 mol $L^{-1}$ with S/L ratio of 20 g $L^{-1}$ at ambient temperature (25-27 °C) conditions. After the leaching step, all the samples were filtered through 0.45 µm filters and analyzed with ICP-OES (iCAP 7600 ICP-OES) to quantify the released concentration of each metal. The method provided by [6] was incorporated to evaluate the rate of release of Pb and Ni from sludge with different concentrations of leaching solvents [Equation 01].

$$R_T = K_T a_{acid}^{n_T} \qquad \text{[Equation 01]}$$

where $R_T$ (mol $m^{-2}$ $s^{-1}$) is the total metal dissolution rate with the reaction of an acid, $K_T$ (mol $m^{-2}$ $s^{-1}$) is the rate constant, $n$ is the experimentally determined reaction factor, and $a$ is the activity of the particular acid, based on the concentration.

Similarly, Equation 02 was utilized for the evaluation of pH dependency for the release rate of total Pb and Ni by a particular acid with the same variables as in Equation 01.

$$logR_T = logk_T - n_T pH \qquad \text{[Equation 02]}$$

To find out proton and ligand-promoted mechanisms, log $R_T$ values for release of each metal were plotted against the pH of acid at particular concentrations used. The $R_T$ for each metal at pH 1 was calculated by Equation 02 using the values obtained for log $K_T$ (intercept) and $n_T$ (slope).

**Results and Discussion**

Table 1 represents the chemical properties and metal composition of battery industrial sludge. Battery industrial sludge composed with Al, Cr, Fe, Pb, Zn, Ti, Mn, and Ni as heavy metals. The heavy metals Pb and Ni were selected to evaluate the leaching rate with different acids as economically important metals that have occurred in considerably high concentrations in battery sludge.

**Table 1.** Chemical properties and elemental composition of battery industrial sludge

| pH (1:10) | EC (mS/cm) | Available N (mg/kg) | Available P (mg/kg) | TOC % | CEC ($cmol_c$/kg) |
|---|---|---|---|---|---|
| 7.27 | 10.78 | 230.01 | 1.35 | 4.79 | 287.85 |

| Element | $Al_2O_3$ | CuO | $Cr_2O_3$ | $Fe_2O_3$ | PbO | $SiO_2$ | ZnO | $TiO_2$ | MnO | NiO |
|---|---|---|---|---|---|---|---|---|---|---|
| w %* | 21.80 | ND | 0.45 | 11.95 | 1.48 | 7.45 | 0.34 | 0.57 | 0.09 | 0.18 |

EC = electrical conductivity, TOC = total organic carbon, CEC = cation exchange capacity, and ND = not detected. Elemental composition was obtained from XRF and represent as a weight percentage (w %) of oxide of the element.

Figure 1 indicates the metal release rates from battery industrial sludge with changing concentrations of each inorganic and organic acid. Nitric acid at more than 0.5 mol $L^{-1}$, caused for the maximum release rates of both Pb and Ni. However, the maximum release rate of Pb ($1.0 \times 10^{-10}$ mol $m^{-2}$ $s^{-1}$) resulted with 1.0 mol $L^{-1}$ nitric solution while 0.5 mol $L^{-1}$ nitric solution facilitated the highest release rates for Ni ($3.0 \times 10^{-11}$ mol $m^{-2}$ $s^{-1}$).

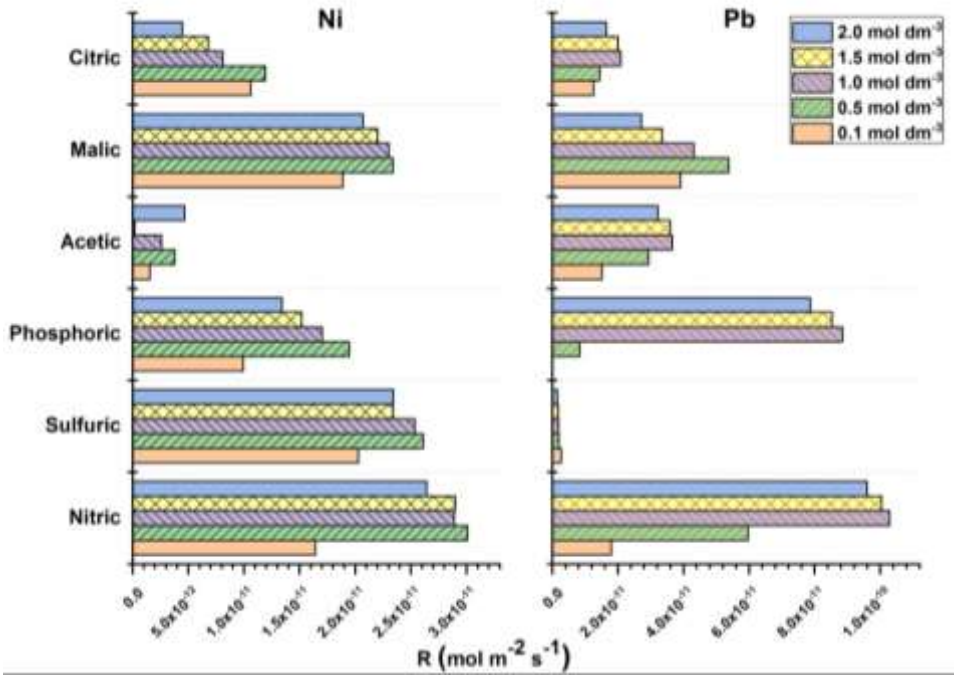


**Figure 1.** Release rates for Ni and Pb from battery industrial sludge by different acids under increasing concentrations.

Previous studies indicated the involvement of proton and ligand promoted factors for the dissolution of metals from minerals and sediments [6]. Ligands

from organic acids have an extensive ability to form metal-ligand complexes that facilitate desorption of metal ions from the matrix [7]. Furthermore, protons from acids dissolve metal ions primarily through ion-exchange. Therefore, the total rate for the release of particular metal ($R_T$) is equal to the combination of rates for proton and ligand promoted release ($R_H$ and $R_L$, respectively) (Equation 03).

$$R_T = R_H + R_L$$

Therefore, Equation 03 used to calculate proton and ligand promoted rates for each acid assuming the ligand promoted rate for nitric acid is zero since, there is no ligand present in nitric acid. Table 2 shows the ligand promoted ($R_L$) and proton promoted rates ($R_H$) for the release of Pb and Ni from battery industrial sludge with different acids at pH 1. The maximum release rate for Pb is achieved with the acetic acid at pH 1 ($R_T$ = 1.90 mol $m^{-2}$ $s^{-1}$ × $10^{-11}$). Contrastingly, the malic acid solution at pH 1 caused for the highest release rate for Ni ($R_T$ = 0.25 mol $m^{-2}$ $s^{-1}$ × $10^{-11}$). Furthermore, comparatively high $R_T/R_H$ ratio for Pb and Ni release respectively with acetic ($R_T/R_H$ for Pb = 8.71) and malic ($R_T/R_H$ for Pb = 1.34) acids indicates the involvement of ligands for release of metals from sludge particles at high rates. It reflects that the complexation of metals with particular ligands provided by organic acids involved facilitating a high rate for release of metals from sludge particles.

**Table 2.** Rate constant ($K_T$), reaction order ($n_T$), total rate of dissolution ($R_T$), ligand-promoted dissolution ($R_L$), and $R_T/R_H$ ratio for Pb, and Ni released from battery industrial sludge with the presence of different inorganic and organic acids.

| Acid | log $K_T$ | $n_T$ | $R_T$ | $R_L$ | $R_T/R_H$ |
|---|---|---|---|---|---|
| | | | mol $m^{-2}$ $s^{-1}$ × $10^{-11}$ | | |
| ***Release of Pb*** | | | | | |
| Nitric | -10.09 | -0.57 | 0.22 | 0.00 | 1.00 |
| Sulfuric | -11.74 | 0.17 | 0.03 | -1.92 | 0.12 |
| Phosphoric | -7.27 | -3.13 | 0.40 | 1.83 | 1.84 |
| Acetic | -9.16 | -0.56 | 1.90 | 16.86 | 8.71 |
| Malic | -10.79 | 0.21 | 0.26 | 0.43 | 1.20 |
| Citric | -10.32 | -0.28 | 0.25 | 0.33 | 1.15 |
| ***Release of Ni*** | | | | | |
| Nitric | -10.56 | -0.16 | 0.19 | 0.00 | 1.00 |
| Sulfuric | -10.62 | -0.05 | 0.22 | 0.28 | 1.15 |
| Phosphoric | -10.60 | -0.21 | 0.15 | -0.34 | 0.82 |
| Acetic | -12.17 | 0.16 | 0.01 | -1.77 | 0.05 |
| Malic | -10.52 | -0.08 | 0.25 | 0.63 | 1.34 |
| Citric | -11.91 | 0.49 | 0.04 | -1.49 | 0.20 |

**Conclusions and Recommendations**

This study indicated that battery industrial sludge was mainly composed of Pb and Ni as economically valuable heavy metals. Acetic acid at pH 1 was involved for highest release rates of Pb from battery industrial sludge and malic acid at pH 1 achieved the maximum rate for release of Ni. The high rates of release of Pb and Ni respectively with organic acids, acetic and malic attributed with formation of metal-ligand complexes which increase the rate of dissolution. Further studies should be carried out with different types of industrial sludge in order to find out exact mechanisms that involve for metal release by acids.

**Acknowledgement**

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# ABUNDANCE AND RICHNESS OF ARBUSCULAR MYCORRHIZAL FUNGI IN *Dicranopteris linearis* DOMINATED LANDS IN BUFFER ZONE OF KANNELIYA FOREST RESERVE, SRI LANKA

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## Introduction

Kanneliya Forest Reserve (KFR) is a lowland rainforest located in the South-western of Sri Lanka. *Dicranopteris linearis* (*'Kekilla'*) has been colonizing forest edges and other disturbed habitats in the buffer zone of KFR. It is an aggressive weed that can form dense thickets, thus preventing the natural regeneration [1]. The anthropogenic disturbances and frequent fires drive *D. linearis* to grow and spread rapidly in areas adjoining forests. The recalcitrant nature of *D. linearis* litter may alter edaphic properties. The removal of *D. linearis* is suggested as the only option to revert these fern lands back to forests [1]. There is strong evidence to suggest that the below-ground resources play an important role in the ecosystem functioning, in particular the arbuscular mycorrhizal fungi (AMF). A higher and diverse population of AMF may enhance the restoration success of degraded lands [1]. It is known that degraded habitats harbor low abundance and diversity of AMF [2], thus acting as one of the constraints of their recovery. Therefore, in order to introduce effective restoration measures, it is important to understand the factors that may limit the recovery of these fern-dominated lands. There is a severe dearth of baseline information on such highly degraded landuse types that could hamper any successful restoration measures to convert these habitats back to forested areas. Thus, the aim of the present study was to evaluate the abundance and diversity of AMF in *D. linearis* dominated fernlands (DD) located in the buffer zone of KFR. The results were compared with an adjoining lowland rainforest (LR) and shrub-dominated site (SD). The findings of this study will provide information on the potential of these fernlands to restore its original status with or without interventions.

## Materials and Methods

From each study site, 20 well represented soil samples were collected from a depth of 0 – 15 cm totaling 60 samples. The soil samples were refrigerated until the analyses. AMF spores were extracted using the wet sieving and decanting method by using 500 µm, 125 µm, 63 µm and 45 µm sieve sizes. [3]. Spores were counted using a dissecting microscope and were identified into different morphotypes based on spore wall characteristics (Figure 1). Air-dried soil samples were analyzed for basic soil properties (colour, texture, pH, moisture, $NO_3^-$-N and $PO_4^{3-}$) using standard methods.

**Results and Discussion**

The abundance, richness and diversity of the AMF was significantly lower in fern-dominated land (DD) than in other two habitats, LR and SD, indicating that the single-species dominance affect the AMF community negatively (Table 1). Principal Component Analysis (PCA) also revealed a clear separation of the three communities. However, the shrub-dominated land (SD) despite experiencing frequent disturbances due to fires, the AMF abundance was more or less similar to that of the adjoining Lowland Rainforest (LR), indicating their resilience to disturbances. The overall results suggest that *D. linearis* dominance has negatively affected the AMF population predicting a poor natural succession even after the fern cover is removed. In favour, previous studies noted that even after years of reforestation, the recovery process is rather slow, thus highlighting the need of assisted restoration measures in order to revert these fern-dominated lands back to forests. *D. linearis* dominated soils showed higher soil pH and organic carbon in comparison to SD and LR (Table 2). However, soil nutrients (P and N) seemed to be low in DD soils, further supporting their degraded nature.

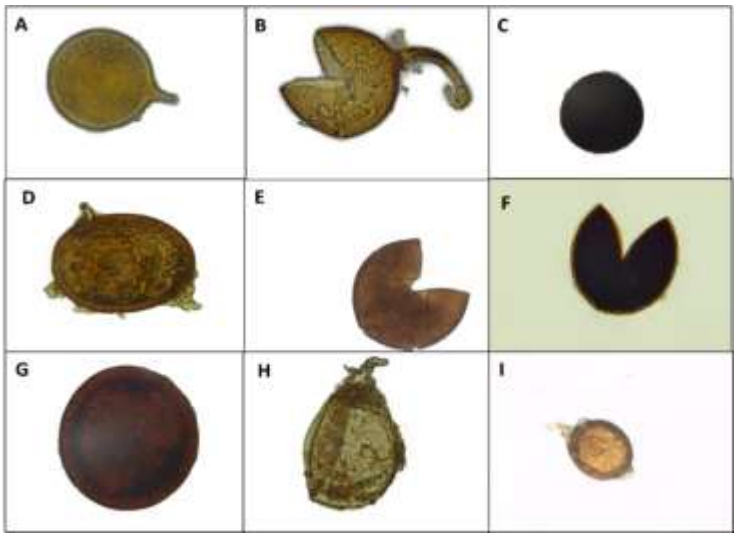


**Figure 1.** Photographs showing AMF spores belong to *Glomus, Acaulospora, Gigaspora, and Scutellospora* species. Photographs were taken from Optika Vision Lite 2.13 (at × 400 magnification) microscope. A-D *Glomus* species, E-F *Acaulospora* species, D-*Gigaspora* sp., H-*Scutellospora* sp. I- unidentified morphotype

**Table 1:** Abundance, richness and diversity indices of arbuscular mycorrhizal fungi (AMF) spores in three landuse types, Lowland rainforest (LR), shrub-dominated site (SD) and *Dicranopteris*-dominated fernland (DD) in the Kanneliya Forest Reserve in the Sri Lanka.

| | LR | | | | SD | | | | *DD* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spore Sizes (µm) | >500 | 125 | 63 | <45 | >500 | 125 | 63 | <45 | >500 | 125 | 63 | <45 |
| Abundance | 2,925 | 2,960 | 3,252 | 2,424 | 1,486 | 2,558 | 3,876 | 3,150 | 1,620 | 1,878 | 1,869 | 838 |
| Richness | 21 | 21 | 21 | 20 | 11 | 12 | 12 | 11 | 7 | 7 | 7 | 6 |
| Total Abundance | 11,561 | | | | 11,070 | | | | 6,205 | | | |
| Total Richness | 21 | | | | 12 | | | | 7 | | | |
| S-W Diversity Index | $2.197^{a}$ | | | | $1.576^{b}$ | | | | $1.339^{c}$ | | | |
| Mean Spore Density (per 100 g fresh soil) | $1,156.1^{a}$ | | | | $1,107.0^{a}$ | | | | $620.5^{b}$ | | | |
| Mean Spore Richness (per 100 g fresh soil) | $20.5^{a}$ | | | | $11.4^{b}$ | | | | $7.0^{c}$ | | | |

The significant differences are highlighted using different superscript letters after one-way ANOVA: Shannon-Wiener Diversity Index n = 10, F = 227.31, p = 0.000; Mean Spore Abundance, n = 10, F = 23.11, p = 0.000; Mean Spore Richness, n = 10, F = 932.67, p = 0.000.

**Table 2.** Soil chemical and physical parameters of the study sites (n = 15), Lowland Rain forest (LR), shrub-dominated site (SD) and *Dicranopteris* dominated fern land (DD) in Kanneliya Forest Reserve in the Sri Lanka.

| Soil parameters | LR | SD | DD |
|---|---|---|---|
| Colour | Very dark brown | Dark brown | Black |
| pH | $4.86^{b}$ | $5.36^{a}$ | $5.27^{a}$ |
| Texture | Clay loam | Clay loam | Clay loam |
| Organic Carbon (%) | 1.3620 | 1.4705 | 1.6875 |
| Soil moisture (g/g) | $0.31^{a}$ | $0.25^{b}$ | $0.28^{ab}$ |
| Phosphorus (µg/g) | $0.505^{a}$ | $0.512^{a}$ | $0.493^{a}$ |
| Nitrate- N (mg/g) | $0.988^{a}$ | $0.750^{b}$ | $0.758^{b}$ |

The significant differences are highlighted using different superscript letters after one-way ANOVA ($F=54.98$, $p\leq0.000$ for soil pH, $P\leq0.01$ for Soil moisture content, $F=0.40$, $P\leq0.671$ for P and $F=31.21$, $P\leq0.000$ for $NO_3^-$ respectively

## Conclusion and Recommendations

The results suggest that single-species dominance in these fernland has negatively influenced the abundance, richness and diversity of AMF. Thus, being one of the most critical factors behind facilitating early plant growth in highly degraded lands, this deprived AMF population may affect the recovery, once the fern cover is removed. Therefore, it is recommended to introduce assisted restoration measures to convert these poor-resource fernlands back to forested areas.

## Acknowledgements

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# FOCUS AREA

## Food, Nutrition and Agriculture

# QUANTIFICATION OF TPC AND TFC OF FLOWERS AND LEAVES OF CHINESE HIBISCUS *(Hibiscus rosa-senesis)* IN SRI LANKA.

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## Introduction

Biochemical and pathophysiological reactions in cells of living organisms result in free radicals causing oxidative stress. This leads to various health problems as aging, cardiovascular diseases and malignancy [1]. Although there is a wide range of antioxidant enzymes within cells, these agents may not be sufficient to normalize the oxidative stress. Under such conditions, supplementation with exogenous antioxidants is necessary to restore the redox homeostasis within cells. Therefore, attempts on developing new antioxidants either of natural or synthetic origin are constantly being researched [1]. However, based on the accumulation of toxic components and side effects present in synthetic antioxidants, it may be more suitable to consume naturally produced antioxidants present in plants [2].

Phytochemicals synthesized as secondary metabolites in plants (as phenolic compounds, flavonoids, stilbenes and proanthocyanidins), represents an important source in therapeutic applications for various diseases and disorders [1, 2]. *Hibiscus rosa-senesis* belonging to the family *Malvaceae* is a perennial plant endemic to Sri Lanka. It is a shrub that consists flowers throughout the year. *H. rosa-senesis* is used as a traditional medicine to treat asthmatic conditions. It is an analgesic, antipyretic and antidiabetic [2]. The plant consists of phytochemical compounds in various parts such as alkanoids, phenols, flavonoids, terpenoids. Leaves and flowers of this plant are used as antimicrobial, antioxidant, antidiabetic, anticancer agents [2].

In Sri Lanka, there were very limited researches carried out on this species related to evaluating its phytochemical contents. Hence, the present study was undertaken to determine the phenolic and flavonoid contents of *H. rosa-senesis* leaves and flowers in different solvent extracts.

## Materials and Methods

Clean undamaged petals and leaves were collected from a home garden, shade dried for 5-6 days, oven dried for 15 seconds at 50° C and ground to obtain a fine powder. Powdered sample of 10g was soaked for 24 hours in 100 mL of the solvent (100% ethanol /100% methanol). The mixture was incubated for 24

hours at room temperature, then filtered using a muslin cloth followed by a No.01 Whatman filter paper. Centrifugation of the filtrate was done at 4000 rpm for 15 minutes and the supernatant was collected. It was evaporated to obtain a solid gel and reconstituted in the same solvent (100% ethanol /100% methanol) to obtain a stock concentration of 40mg/ml. The extract was kept at 4°C till further use [3]. A working concentration of 40mg/ml was prepared to determine the phytochemical content and antioxidant activity using the solvents (100% ethanol /100% methanol).

The Total phenolic content (TPC) was determined using Folin-Ciocalteau method described by [4]. A 10-fold diluted Folin-Ciocalteau reagent (0.5ml) was mixed with 1ml of sample extract and4ml of 7.5% (w/v) $Na_2CO_3$. The mixture was incubated for 10 minutes at room temperature and the absorbance was measured at 765nm against the blank containing solvent (ethanol 100% / methanol 100%). Total phenolic content was determined as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve.

The Total flavonoid content (TFC) was determined by the $AlCl_3$ method [5]. Distilled water (2000µl) was mixed with 100µl of sample extract and 300µl of 5% $NaNO_3$ (w/v). To this, 300µl of 10% $AlCl_3$ (w/v) was added followed by 200µl of 20% NaOH (w/v). The mixture was incubated for 15 minutes at room temperature and the absorbance was measured at 420 nm against the blank containing solvent (ethanol 100% / methanol 100%). Total flavonoid content was determined as mg of rutin equivalent per gram using the equation obtained from a standard rutin calibration curve. All assays were performed in triplicates and the mean and standard deviation was obtained. Statistical analysis was performed using SPSS Statistics 21. ANOVA was used to test the significant among the different samples and t test was performed to determine the significant between samples. The significance was determined at $P<0.05$.

## Results and Discussion

**Table 1**. Total phenolic content (TPC) and total flavonoid content (TFC) in *Hibiscus rosa-senesis* leaves and flower extracts

| Solvent | | TPC (mg GAE / g) | | TFC (mg Rutin /g) | |
|---|---|---|---|---|---|
| | | 20mg/ml | 40mg/ml | 20mg/ml | 40mg/ml |
| 100% Ethanol | leaves | 1.87 ± 0.001 | 13.98 ± 0.0 11 | 177.67 ± 0.008 | 186.68 ± 0.009 |
| | flower | 35.82 ± 0.129 | 44.65 ± 0.025 | 61.59 ± 0.012 | 78.22 ± 0.013 |
| 100% Methanol | leaves | 33.86± 0.047 | 37.37 ± 0.019 | 256.25 ± 0.004 | 160.88 ± 0.019 |
| | flower | 4.9 ± 0.004 | 20.94± 0.006 | 16.17 ± 0.005 | 71.70± 0.007 |

Data represented as mean ± SE (n=3).

According to Table 1, the TPC of *H. rosa-senesis* species at 20 mg/ml was high in the ethanol flower extract followed by the methanol leaf, methanol flower and ethanol leaf extract (P=0.000; P<0.05). The ethanol flower extract demonstrated a higher TPC compared to the ethanol leaf extract (P=0.007; P<0.05), whereas the methanol leaf extract demonstrated a higher TPC compared to the methanol flower extract (P=0.166; P>0.05).

A similar result was observed for the species at 40 mg/ml, where the ethanol flower extract demonstrated a high TPC followed by methanol leaf, methanol flower and ethanol leaf extracts (P=0.000; P<0.05) (Table 1). The ethanol flower extract showed a higher TPC compared to the ethanol leaf extract (P=0.057; P<0.05), whereas the methanol leaf extract showed a higher TPC compared to the methanol flower extract (P=0.166; P>0.05) (Table 1). These observations were in agreement with a research published by [3] and [2], where it clearly states that ethanolic extraction gives an efficient phytochemical content in *H. rosa senesis* flowers and methanolic extract in leaves. However, in this study the observation is only applicable for the total phenolic content.

The total flavonoid content of *H. rosa-senesis* species at 20 mg/ml was high in the methanolic leaf extract followed by the ethanolic leaf, ethanolic flower and methanolic flower extracts (P=0.00; P<0.05). The ethanolic leaf extract demonstrated a higher TFC compared to the ethanol flower extracts (P=0.013; P<0.05). Similarly, the methanolic leaf extract demonstrated a higher TFC compared to the methanolic flower extract (P=0.00; P<0.05).

The TFC of *H. rosa-senesis* species at 40 mg/ml showed a high content in the ethanolic leaf extract followed by the methanolic leaf, ethanolic flower and ethanolic flower (P=0.000; P<0.05). The ethanolic leaf extract showed a higher TFC compared to the flower extract (P=0.002; P<0.05). Similarly, the methanolic leaf extract showed a higher TFC compared to the flower extract (P=0.055; P>0.05) (Table 1). According to a study, it was found to observe the presence of flavonoids in leaves than in flowers of *H. rosa-senesis* [5]. All extracts demonstrated a higher TFC value compared to the TPC value at both concentrations (ethanol leaf: (P=0.055; P>0.05); ethanol flower: (P=0.070; P>0.05) methanol leaf: (P=0.907; P>0.05); methanol flower: (P=0.000< P=0.05) (Table 1).

**Conclusion and Recommendations**

As per the current study, *H. rosa-senesis* leaves demonstrated a higher flavonoid content compared to the flowers in both solvent extracts. Further, it may be suggested that Methanol is a better extraction medium for *H. rosa-senesis* leaves and ethanol is a better extraction medium for *H. rosa-senesis* flowers to obtain a high content of phenols and flavonoids.

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# SCREENING OKRA (*Abelmoschus esculentus*) GERMPLASM FOR YELLOW VEIN MOSAIC VIRUS DISEASE RESISTANCE

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## Introduction

Okra (*Abelmoschus esculentus*) is an important vegetable crop of family Malvaceae. It has become a popular vegetable crop among farmers since it can be cultivated in both seasons and generate an early income. This crop is suitable for home gardening and large scale commercial cultivation. Okra fruits are rich in vitamin C, folate, calcium and potassium and it is a good source of fiber and antioxidants. There are some unique medicinal properties in okra. The special fibers contained in okra has the ability of controlling blood sugar level, provide purgative property and relief hemorrhoids [1].

Origin of the okra is reported to be in African or Asiatic region or both regions. It is cultivated in tropical and subtropical areas such as Asia, Africa, and America. The plant is an annual herb, erect, robust and ranging between 1 to 2 m in height with simple leaves. Flowers are hermaphrodite, regular and sole. The fruit is a pod, 10 to 30 cm long, pubescent or glabrous with ridges, in variable color. Anthesis takes place before flower open, so conditions are favorable for self-pollination. Pods develop rapidly in about 11 days and harvesting can be done 4-10 days after flowering. [2]

Okra is primarily cultivated for its edible immature pods, but young leaves and mature seeds are also eaten as vegetables in most African countries. Okra cultivation is profitable especially in monocropping system, but also can cultivate as intercropping. Okra is one of the most popular fruit vegetable grown in Wet, Intermediate and Dry zone of Sri Lanka and there are few recommended varieties including *Haritha*, *MI – 5* and *MI – 7.*

Diseases such as Yellow Vein Mosaic Virus (YVMV), damping-off, vascular wilt, powdery mildew and the pests including fruit and stem borers (*Earias spp., Heliothis spp.),* flea beetles (*Podagrica spp.)* and white fly (*Bemisia tabaci*) are the major problems in okra cultivation. Among them YVMV is a serious problem that causes yield losses ranging from 50% to 94% depending upon growth stage of the plant. The amount of damage declines with delay in infection the plants. The Okra mosaic virus is characterized by a network of yellow mosaic patterns that encompass green tissue islands in leaf blades. The fruits of the infected

plants are pale yellow to white, deformed, small and hard in texture. The virus is mainly transmitted by the beetles of *Podagrica spp*. and white fly. Vectors usually attack the young okra plants at the vegetative stage [3].

A virus infection cannot be completely cured once the plant is infected. Use of healthy cultural practices and the vector control are the agronomic approaches in controlling the disease. However, the development of improved okra varieties resistant to the YVMV is the only long term approach to face this challenge. Availability of virus resistant germplasm is a prerequisite for undertaking the hybridization programs in improving the crop. Thus, the present study was conducted to screen the okra germplasm for YVMV in order to select the potential resistant genotypes.

## Materials and Methods

*Field establishment*

The research was conducted at the field of Landmark Agro Seed Company Maho, Sri Lanka from July to October 2019. Two blocks each with 28 plots (1.5×4.0 m) were arranged to minimize the shade effect. Five okra Varieties (*Haritha, MI 05, Sakura, Vimukthi, Shakthi*) and 23 accessions were collected from local and exotic germplasm. Thirty plants were maintained from each germplasm. All the agronomic practices were done according to the recommendation of DOA. The plants were inoculated with the virus.

*Virus Inoculation*

Infected okra leaves were collected from different parts of the country including Hambantota, Kurunegala, Gampaha, Anuradhapura and Puttalam districts and transported to the field in an ice box. The extraction was prepared by grinding the infected okra leaves in distilled water. Inoculation was commenced one month after seeding and continued at every ten days up to six applications. First inoculation was done using spray method and the other three applications were done by injecting 2 ml of extract using a disposable syringe with 21G×11/2" needle. Last two inoculations were done by spraying on to the gently rubbed leaf blade using 400 gauge sand paper.

*Estimating disease severity*

Severity of the YVMV disease symptoms in infected plants were calculated using a formula published by Galanihe [4].

$$\text{Disease Severity Index (DSI) \%} = \frac{\sum (V \times n)}{N \times Z} \times 100$$

Where, V = Severity score, n = Number of affected plants having same score, N = Total number of plants observed, and Z = Maximum scale number.

Severity scale and the description of damage is given below.

0 - No disease symptoms
1 - <10% of the canopy showing disease symptoms
3 - 10-25% of the canopy showing disease symptoms
5 - 25-50% of the canopy showing disease symptoms
7 - 50-75% of the canopy showing disease symptoms
9 - >75% of the canopy showing disease symptoms

*Estimating disease incidence*

Disease Incidence (DI %) was calculated using a formula published by Banerjee and Kalloo [5].

$$\text{DI \%} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

Disease severity index and disease incidence was categorized in to five categories based on the frequency of severity grades in given (Table 1) [5].

**Table 1.** Disease resistance based on the Disease Incidence (DI)

| YVMV Symptoms | Severity Grade | Reaction |
|---|---|---|
| Symptoms absent | 0 | Highly Resistance |
| Very mild symptoms up to 25% plant | 1 | Resistance |
| Appearance of symptoms in 26-50% plant | 2 | Moderately Resistance |
| Appearance of symptoms in 51-75% plant | 3 | Moderately susceptible |
| Sever disease infection in symptoms (>75% plant) | 4 | Highly susceptible |

*Experimental design and data re cording*

Randomized Complete Block Design (RCBD) was used for the study. From each genotype in each block 15 plants were randomly selected and labeled for recording the data. Diseased infection was recognized in the emerging leaves having Yellow vain mosaic symptoms. The plants were closely observed and data recording was started two weeks after the first inoculation and continued in every two weeks intervals. Total number of leaves and number of infected leaves were recorded as the vegetative parameters in every two weeks. Number of flowers and pods were recorded as a reproductive parameters at same intervals.

*Data analysis*

The recorded data were analyzed using the Minitab software. Screen out test was done using ANOVA.

**Results and Discussion**

A total of 28 okra genotypes were categorized in to five groups (Table 2) based on the resistance estimated in two ways of DSI and DI. According to the DSI and

DI categorization two okra germplasm (*Bhindi 2016 03*, *Bhindi 2016 04*) were highly resistant for the virus. These two accessions did not show any symptoms development throughout the study. Ten gernotypes including 3 varieties (*Sakura, Vimukthi, Shakthi*) and other genotypes (*Bhindi indus 101, Bhindi indus 121, BH 2015 17, BH 2015 34, Bhindi 2015 18, Bhindi F1, Maha F1*) belongs to resistance group. Rest of the Varieties and accessions belongs to groups of moderately resistance (*Bhindi Indus 101*, *BH 04*, *BH 13*, *BH 2015 33*, *Prawin 999*), moderately susceptible (*Haritha*, *BH 1010*) and highly susceptible

**Table 2.** Okra accessions and varieties grouped according to the severity grades

| Variety/ Accession | DSI | DI |
|---|---|---|
| | Reaction | Reaction |
| (1) *Athdala okra* | 4 | 4 |
| (2) *Sarath okra* | 2 | 4 |
| (3) *Athdala okra 5042* | 4 | 4 |
| (4) *Red okra* | 3 | 4 |
| (5) *Haritha* | 2 | 3 |
| (6) *MI 05* | 2 | 4 |
| (7) *Bhindi Indus 101* | 1 | 2 |
| (8) *Bhindi Indus 111* | 1 | 1 |
| (9) *Bhindi Indus 121* | 1 | 1 |
| (10) *BH 04* | 1 | 2 |
| (11) *BH 13* | 1 | 2 |
| (12) *BH 2015-17* | 1 | 1 |
| (13) *BH 2015-33* | 1 | 2 |
| (14) *BH 2015-34* | 1 | 1 |
| (15) *BH 2015-72* | 2 | 4 |
| (16) *Bhindi BH 101* | 3 | 4 |
| (17) *Bhindi 2016-03* | 0 | 0 |
| (18) *Bhindi 2016-04* | 0 | 0 |
| (19) *BH 1010* | 2 | 3 |
| (20) *Bhindi 2015-18* | 1 | 1 |
| (21) *Green finger* | 3 | 4 |
| (22) *Prawin 999* | 1 | 2 |
| (23) *Bhindi sumithra* | 3 | 4 |
| (24) *Sakura* | 1 | 1 |
| (25) *Vimukthi* | 1 | 1 |
| (26) *Shakthi* | 1 | 1 |
| (27) *Bhindi F1* | 1 | 1 |
| (28) *Maha F1* | 1 | 1 |

The development of disease symptom of seeding was observed from 6th week onwards. Even though it has been reported that YVMV is neither sap transmissible nor seed transmissible, it was observed that the symptoms were developed after application of the virus. However, during the latter part of the study heavy white fly population was observed in the field that caused natural transmission of the virus. Four highly susceptible genotypes were proven to infection at very early stage of plant development. About 53.5% genotypes

showed disease development after 8 weeks of seeding. After 10 weeks of seeding the highest disease spreading was observed within the genotype. The difference in the stage of symptom development among the varieties could be attributed with the inoculated virus strain. Different virus strains are available in different parts of the country. At each inoculation the virus inoculum was prepared using the infected leaves collected from different part of the country. Thus based on the susceptibility of the variety for the specific strain the time taken for the disease development could be varied. However, after 6th application the disease spreading ability was achieved to its maximum. *Bhindi 2016 03* and *Bhindi 2016 04* the highly resistant genotypes could be considered as the resistant for all types of virus strains inoculated.

Disease incidence and infection rate of each genotype can be identified (Figure 1).

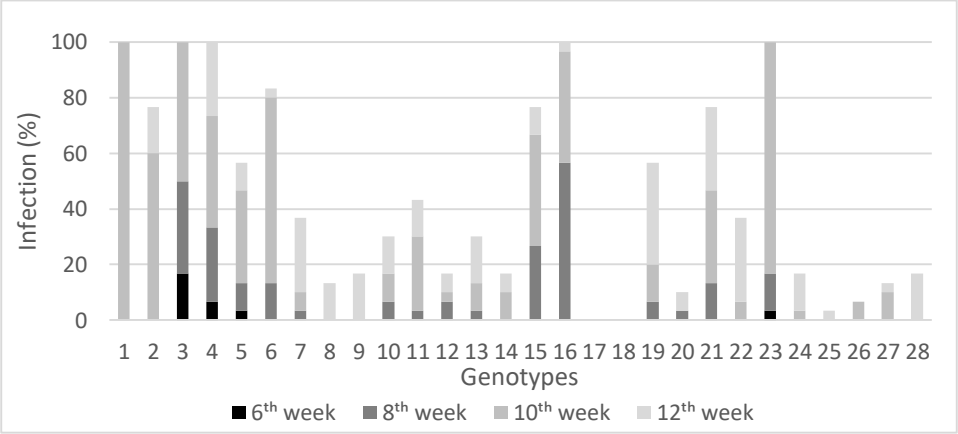


**Figure 1**. Disease infection rate of the evaluated okra germplasms after inoculating the virus at different time periods

**Conclusions and Recommendations**

Among the 23 accessions and 5 varieties, seven accessions (*Bhindi indus 101, Bhindi indus 121, BH 2015 17, BH 2015 34, Bhindi 2015 18, Bhindi F1, Maha F1*) and three varieties (*Sakura, Vimukthi, Shakthi*) showed that they are resitant to YVMV. Another two accessions of (*Bhindi 2016 03, Bhindi 2016 04*) showed highly resistance to the YVMV. These nine okra accessions and 3 varieties are suitable for further screening of YVMV resistance in other seasons and at different locations. These germplasms can be used for hybridization programs to develop new okra varieties resistance to YVMV. *Sakura, Vimukthi* and *Shakthi* are recommended to cultivation as resistance varieties.

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# COMPARATIVE STUDY ON THE OXIDATIVE STABILITY OF COCONUT OIL AND SESAME OIL AND THEIR BLENDS

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## Introduction

Studies on edible oil become one of the prime areas of research. Edible oils serve as important source of dietary lipids. These are widely used as cooking oils and as an ingredient in variety of foods. Major deteriorative reaction occurs in most of the edible oils is oxidation leading to the production of various primary and secondary compounds, which have negative impacts on nutritional and sensory qualities of oils. Oxidative stability of oils is defined as the resistance to oxidation during processing and storage and it occurs by several molecular mechanisms such as autoxidation, photosensitized oxidation and thermal oxidation. Lipid oxidation products are considered to be harmful for health as they consist of compounds with mutagenic, carcinogenic and cytotoxic properties. Thus, it is necessary to improve the oxidative stability of oils.

Different methods are being used to improve the stability of oils against oxidation such as use of antioxidants, blending of two or more oils, natural plant breeding and genetic modification. Blending of different oils modifies the physicochemical properties of oils without changing their chemical composition. It is recommended that intake of oil should contain saturated, monounsaturated and polyunsaturated fatty acids in the ratio 1:1:1 [1, 6]. Thus, blending can be used to improve the oxidative stability as well as nutritional quality of edible oils.

In Sri Lanka, coconut oil and sesame oil are the widely used edible oils. Coconut oil is highly stable to oxidation due to its high level of saturation, whereas Sesame oil contains high amount of mono and poly unsaturated fatty acids, which have beneficial effects on human health, however, it is less stable for oxidation than coconut oil during heat processing. Thus, making blend using coconut oil and sesame oil is beneficial to overcome the limitation and combine benefits of both oils. In this backdrop, this study was aimed to evaluate the oxidative stability and nutritional property as the ratio of saturated: monounsaturated: polyunsaturated fatty acids of coconut oil, sesame oil and their blends.

## Materials and Methods

*Materials*

Sesame oil and coconut oil were purchased from mills located in Jaffna. It was ensured that oils were free of any synthetic antioxidant and stored at -18 °C until use within a week.

*Preparation of oil blends*

Three oil blends with different proportions of coconut and sesame oils were prepared as indicated in the Table 1 for testing along with the raw oils as references. Oils were taken in required quantity in a conical flask and mixed thoroughly using a magnetic stirrer for 15 minutes.

**Table 1.** Blends of coconut oil and sesame oil

| Blend No | Ratio of coconut oil: sesame oil (v/v) |
|---|---|
| Blend 1 | 70:30 |
| Blend 2 | 50:50 |
| Blend 3 | 30:70 |

*Accelerated oven storage test*

Accelerated oven storage test was conducted according to AOCS Official method Cg 5-97. Briefly, 4 mL of each oil samples was added in to clear glass vials (5 mL). All samples were prepared in four sets with the same procedure. Then the samples were capped loosely and stored in an oven (Memmert) maintained at the temperature of 60 ±5 °C for up to 14 days.

*Analysis of oil*

One set of each samples were taken on 1$^{st}$, 3$^{rd}$, 7$^{th}$ and 14$^{th}$ day of storage and analyzed for the level of oxidation by determining free fatty acid content [2], peroxide value [3] and *p-Ansidine* value [2].

*Determination of fatty acid profile using Gas-Liquid chromatography*

FAMEs were prepared according to ISO 12966-2:2011 [4]. Fatty acid profile of the samples was analyzed using Gas-Liquid Chromatography.

*Statistical analysis*

Analysis of variance (ANOVA) was calculated using the two factors Completely Randomized Design using Statistical Analysis System (SAS), version 9.1.3. Duncan's Multiple Range Test was used to compare the treatment means at $p<0.05$ using IBM SPSS Statistics 20.

## Results and Discussion

*Initial quality of oils*

Initial free fatty acid content of coconut oil and sesame oil were 0.32 % (% of lauric acid) and 2.35 % (% of oleic acid), respectively. Peroxide value of coconut and sesame oils were 0.6 and 2.0 meq/kg, respectively. According to Sri Lankan Standard, the maximum free fatty acid content of coconut oil and sesame oil should be 0.8% (lauric acid) and 3% (oleic acid) respectively [5]. Peroxide value for refined oil should be less than 10 meq/kg of sample. Thus, initial quality of oils was assured.

*Changes in chemical properties*

*Free fatty acid content*

Free fatty acid content is an indicator of keeping quality of oil. From initial to 14 days of storage, raw oils as well as their blends conform to the Sri Lankan Standard. Free fatty acid content of coconut oil increased from 0.33% to 0.59%, while free fatty acid content of sesame oil increased from 2.37% to 2.94%. Thus, it is obvious that free fatty acid content of blends increased with the addition of sesame oil, because initial free fatty acid content of sesame oil is higher than that of coconut oil.

*Peroxide value*

Peroxide value of raw oils and their blends recorded during the fourteen days storage under accelerated oven storage are presented in Table 2. From initial to fourteen days of storage, raw oils and their blends conform to Sri Lankan Standard. Sesame oil showed highest peroxide value, while, blend 1 showed lowest peroxide value than other blends. Relatively low peroxide values were recorded in the blends containing high percentages of coconut oil (100 % to 70% coconut oil).

*p-Anisidine value*

*p*-Anisidine value of raw oils (coconut oil and sesame oil) and their blends recorded during the fourteen days storage under accelerated oven storage are presented in Table 2. Anisidine value test is commonly used to assess the secondary oxidation of oils which is mainly imputable to aldehydes and ketones. Initial *p*-Anisidine value of sesame oil and coconut oil were 0.3 and 0.04, respectively. Blends of sesame oil and coconut oil having *p*-Anisidine value are greater than coconut oil and less than sesame oil. Increasing of *p*-anisidine value observed with the increasing amount of sesame oil.

**Table 2.** Free fatty acid content, peroxide value and p-anisidine value of oil samples

| Samples | 0 | 1 | 3 | 7 | 14 |
|---|---|---|---|---|---|
| | | | **Free fatty acid content** | | |
| SO | 2.37±0.02$^{Ae}$ | 2.49±0.02$^{Ad}$ | 2.54±0.04$^{Ac}$ | 2.71±0.05$^{Ab}$ | 2.94±0.09$^{Aa}$ |
| CO | 0.33±0.02$^{Ee}$ | 0.47±0.02$^{Ed}$ | 0.56±0.02$^{Ec}$ | 0.57±0.02$^{Eb}$ | 0.59±0.04$^{Ea}$ |
| B1 | 0.98±0.01$^{De}$ | 1.04±0.01$^{Dd}$ | 1.08±0.02$^{Dc}$ | 1.22±0.01$^{Db}$ | 1.30±0.07$^{Da}$ |
| B2 | 1.38±0.01$^{Be}$ | 1.48±0.02$^{Cd}$ | 1.49±0.02$^{Cc}$ | 1.48±0.01$^{Cb}$ | 1.56±0.04$^{Ba}$ |
| B3 | 1.69±0.02$^{Ce}$ | 1.78±0.03$^{Bd}$ | 1.96±0.04$^{Bc}$ | 1.99±0.01$^{Bb}$ | 2.05±0.01$^{Ca}$ |
| | | | **Peroxide value** | | |
| SO | 1.79±0.02$^{Ae}$ | 1.81±0.01$^{Ad}$ | 2.01±0.02$^{Ab}$ | 3.13±0.04$^{Ac}$ | 6.55±0.03$^{Aa}$ |
| CO | 0.08±0.03$^{Ee}$ | 0.09±0.02$^{Ed}$ | 0.10±0.04$^{Ec}$ | 0.14±0.03$^{Eb}$ | 0.22±0.02$^{Ea}$ |
| B1 | 0.98±0.04$^{Dd}$ | 1.28±0.01$^{Dd}$ | 1.51±0.03$^{Dc}$ | 1.74±0.04$^{Db}$ | 2.49±0.02$^{Da}$ |
| B2 | 1.06±0.01$^{Be}$ | 1.13±0.01$^{Bd}$ | 2.58±0.02$^{Bc}$ | 3.17±0.08$^{Bb}$ | 3.96±0.05$^{Ba}$ |
| B3 | 1.37±0.02$^{Ce}$ | 1.39±0.01$^{Cd}$ | 1.73±0.02$^{Cc}$ | 2.21±0.03$^{Cb}$ | 3.02±0.06$^{Ca}$ |
| | | | **p-anisidine value** | | |
| SO | 0.33±0.01$^{Ad}$ | 1.62±0.02$^{Ac}$ | 2.13±0.05$^{Ac}$ | 2.72±0.06$^{Ab}$ | 3.03±0.04$^{Aa}$ |
| CO | 0.04±0.01$^{Ed}$ | 0.12±0.05$^{Ec}$ | 0.34±0.02$^{Ec}$ | 0.39±0.05$^{Eb}$ | 0.80±0.04$^{Ea}$ |
| B1 | 0.05±0.01$^{Dd}$ | 0.07±0.02$^{Dc}$ | 0.10±0.04$^{Dc}$ | 0.18±0.02$^{Db}$ | 0.32±0.01$^{Da}$ |
| B2 | 0.07±0.01$^{cd}$ | 0.09±0.05$^{Cc}$ | 0.12±0.06$^{Cc}$ | 0.23±0.01$^{Cb}$ | 0.58±0.06$^{ca}$ |
| B3 | 0.12±0.02$^{Bd}$ | 0.98±0.03$^{Bc}$ | 1.23±0.04$^{Bc}$ | 2.01±0.03$^{Bb}$ | 2.75±0.02$^{Ba}$ |

SO=Sesame oil , CO=Coconut oil, B3=Blend 1(70% coconut oil and 30% sesame oil) ,B2=Blend 2(50% coconut oil and 50% sesame oil) , B1=Blend 3(70% sesame oil and 30% coconut oil)
Values are means ± standard deviation for duplicate analyses.
Means in each row followed by different superscripts letters (a-e) are significantly different (P<0.05). Means in each column followed by different superscripts letters (A-E) are significantly different (P<0.05).

*Fatty acid composition*

Percentage content of the saturated, monounsaturated and polyunsaturated fatty acids in the raw oils and oil blends are presented in Table 3. Blend 1 showed SFA: MUFA: PUFA in the ratio of 6:1:1 although it shows highest oxidative stability than other blends. Blend 3 had achieved the ratio of 1:1:1.

**Table 3.** Fatty acid composition of raw oils and their blends

| | Oil samples | | | | |
|---|---|---|---|---|---|
| **Fatty acid** | **SO** | **Blend 3** | **Blend 2** | **Blend 1** | **CO** |
| SFA | 15.91 | 38.39 | 57.48 | 74.14 | 92.24 |
| MUFA | 37.78 | 28.62 | 20.63 | 13.65 | 6.27 |
| PUFA | 46.31 | 32.99 | 21.89 | 12.21 | 1.49 |
| SFA:MUFA:PUFA | 1:2:3 | 1:1:1 | 3:1:1 | 6:1:1 | 62:4:1 |

SFA – Saturated fatty acids; MUFA – Monounsaturated fatty acid; PUFA – Polyunsaturated fatty acids.

**Conclusions and Recommendations**

It is found that blend having 70% of coconut oil and 30% of sesame oil (blend 1) exhibited higher oxidative stability than other blends with higher amount of sesame oil. Based on the fatty acid composition (proportion of saturated: monounsaturated: polyunsaturated fatty acids), blend 3 had achieved the ideal ratio of 1:1:1. However, it exhibited poor oxidative stability compared to other two blends. Therefore, it can be concluded that blend I can be used for processing application using high heat (such as frying) and blend 3 can be used for low heat applications.

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# BIOTIC RISKS, PESTICIDE USAGE AND AWARENESS ON IPM TECHNIQUES IN PADDY CULTIVATION IN POLONNARUWA DISTRICT OF SRI LANKA

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## Introduction

Rice (*Oryza sativa*) is one of the most important staple foods for most of the world population [1] including Sri Lanka. The sown extent of rice cultivation and production during 2017/2018 *Maha* season in Sri Lanka was estimated as 667,191 ha and 2,396,926 MT respectively [2] and rice provides 45% of total calorie and 40% of total protein requirement of an average Sri Lankan [3]. Nowadays, usage of pesticides in rice cultivation has been promoted among farmers in developing countries including Sri Lanka to increase the productivity. Rapid increase in the application of pesticides has posed threats [4] as the usage of weedicides, insecticides, fungicides and fertilizers have been associated with negative environmental and potential health hazards to humans [5, 6].

Polonnaruwa, a cultural metropolis in the North Central Province of Sri Lanka, falls under the dry zone climatic conditions is the second highest paddy producing district next to Ampara district in Sri Lanka [2]. There are seven divisional secretariat (DS) divisions in Polonnaruwa district, namely Thamankaduwa, Hingurakgoda, Lankapura, Madirigiriya, Elahera, Dimbulagala and Welikanda. Farmers in these areas experience different types of risk exposures during the cultivation such as weeds, insect pests, diseases, etc. To manage these problems, farmers chiefly use chemical pesticides. They practice more number of applications and use high dosage of pesticides. Based on this background, this study was undertaken in 2018 to assess the risk exposures in paddy cultivation in this area, to study the pesticide usage in paddy cultivation and to evaluate coping mechanisms used for pest management.

## Materials and Methods

A questionnaire survey was conducted using stratified random sampling from Thamankaduwa, Hingurakgoda, Lankapura, Madirigiriya, Elahera, Dimbulagala and Welikanda DS Divisions in Plonnaruwa district (Figure 1) and the final sample comprised of 50 paddy farmers. Structured questionnaires designed to gather required information and they were pretested to assess their suitability. Selected respondents were interviewed at their farming sites and field observations were also made. Data were subjected to descriptive statistics and correlation analysis was done using SPSS statistical software version 19.0.

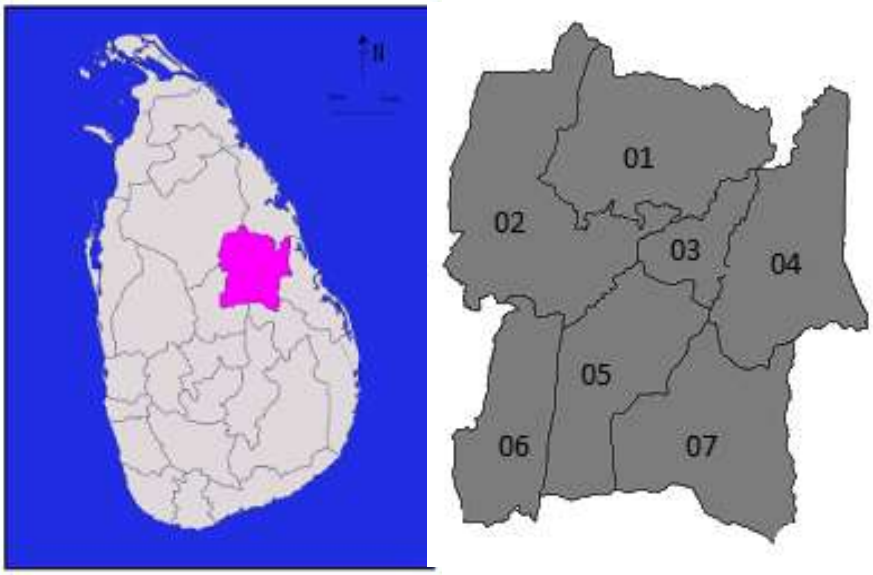


**Figure 1**. Sri Lankan map showing DS Divisions of Plonnaruwa district, Sri Lanka (1-Medirigiriya 2-Hingurakgoda, 3- Lankapura, 4- Welikanda, 5- Thamankaduwa, 6- Elahera 7- Dimbulagala)

## Results and Discussion

*Results related to socio-economic status of the paddy farmers*

The results of the socio-economic variables of paddy farmers in Pollonaruwa district revealed that average age of farmers was 47 years and average family size was 5. Most of the farmers (50%) had education level grade 6 to grade 11. The data showed that 82% of the farmers had more than 5 years of experience while no farmers had less than 1 year of experience in paddy cultivation. Fifty-four percent of the farmers were engaged in full time paddy cultivation. The average extent of cultivation was 5.27 Ac. Average monthly income of the selected farmers was 44,700 Sri Lankan Rupees.

*Results related to potential biotic risks and coping mechanism*

The results related to the potential biotic risks identified and the coping mechanisms followed by the farmers in Polonnaruwa district during paddy cultivation are listed in the Table 1 given below. Weed infestation and insect pest attack were identified as the potential biotic stresses for the paddy cultivation in Polonnaruwa district.

**Table 1**. Results related to potential biotic risks faced by the farmers and coping mechanism in paddy cultivation in Polonnaruwa district, Sri Lanka in 2018.

| S/N | Attributes | Categories | Percentage |
|---|---|---|---|
| 1 | Potential biotic risks | Weeds | 96% |
| | | Insect pests | 4% |
| | | Diseases | 0% |
| 2 | Coping mechanisms | Using agro chemicals | 100% |
| | | Using resistant cultivars | 0% |
| | | Optimum time cultivation | 0% |
| | | Bio pesticides | 0% |
| | | Trap crops | 0% |
| | | Using cover crops | 0% |
| | | Using mulch | 0% |
| | | Crop rotation | 0% |
| 3 | Weedicides | Pretilachlor | 100% |
| | | MCPA | 26% |
| | | 3,4 -DPA | 18% |
| 4 | Insecticides | Imidacloprid | 22% |
| | | Abamectin | 20% |
| | | Carbosulfan | 12% |

S/N: Serial number, MCPA; 2-methyl-4-chlorophenoxyacetic acid, 3,4 -DPA; 3,4-diphenylamine

Ninety-six percent of the farmers stated weed infestation as the major biotic stress, while insect pest attack was only identified by 4% of the respondents in the paddy cultivation. Remarkably, there were no reports made on disease damages in paddy cultivated in Polonnaruwa district in 2018. Majority of the farmers (72%) stated that dominant weed that affected their cultivation was *Ischaemum rugosum* Salisb and other weeds such as *Echinocloa glabrescens* Munro, *Scripus supines* L. and *Isachne globoa* Okuntze also affect the cultivation at a % of 48, 46 and 44%, respectively.

Synthetic chemicals were commonly used by the farmers in order to cope up the biotic problems of the rice cultivation. The study showed that all the farmers (100%) used agrochemicals for their paddy cultivation in Polonnaruwa district. Among the weedicides, Pretilachlor, a broad spectrum weedicide was applied by all the farmers. In addition, 26% of farmers apply MCPA and 18% use 3, 4-DPA in order to control the weeds. Insecticides are used by less than 26% of the farmers in Polonnaruwa district. Among them, imidacloprid was mostly applied (22%) followed by abamectin (20%) and carbosulfan (12%) in order to manage the insect pest attacks in their paddy fields.

*Precautionary measures*

The precautionary practices adopted by the farmers are given in the Figure 2.

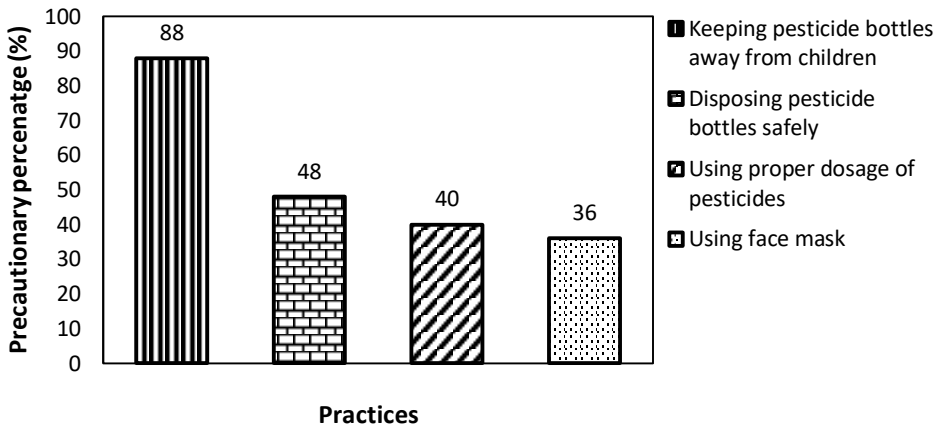


**Figure 2.** Percentage of farmers practicing different precautionary measures

In the study area, 88% of the farmers keep the chemical bottles away from children and 48% of them dispose chemical bottles safely. It is noted that some of them (40%) use the proper dosage of chemicals, while 36% of them use face mask for their safety as shown in the Figure 2. It seems that the farmers generally follow less precautionary methods.

*Health problems faced by farmers*

According to the gathered information usage of chemicals has caused severe problems to farmers. Mainly respiratory disorders were reported by 60% of the farmers and around 30 and 10% have been suffered with headache and faintness, respectively.

*Preference towards Integrated Pest Management (IPM) and Source of Information about IPM*

Besides using agrochemicals, 80% of the farmers prefer to practice IPM. Most of the farmers (68%) find information about IPM from the Department of Agriculture, Sri Lanka. About 44% of farmers collect information on IPM from Agrarian Service Centres and a few (10%) obtains details from their neighboring farmers.

*Results of correlation analysis*

Correlation analysis showed a significant positive correlation ($R^2$=0.82; P=0.028) of farmers' income with their extent of paddy cultivation. Furthermore, farmers' knowledge on IPM had a significantly positive correlation ($R^2$=0.76; P=0.034) with their education level.

**Conclusions and Recommendations**

Weed infestation was the predominant biotic risk experienced by the farmers in the Polannaruwa district. All the farmers are dependent on chemical pesticides to control weeds and the insect pests. Pretilachlor weedicide was used by all the farmers surveyed. In general, the paddy farmers follow less precautionary measures with regard to pesticide applications. Extent of paddy cultivation and farmers' educational level had significant influence on farmers' income and their knowledge on IPM, respectively. It is recommended to increase the effectiveness of extension services, motivate farmers for better use of pesticides through generating awareness about ill effects of misuse of chemical pesticides and provide farmer training on alternative aspects of pest control strategies such as IPM.

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# SEEDLING TRANSFORMATION OF *Anthurium andraeanum* Linden Ex Andre USING *Agrobacterium tumefaciens* CARRYING A *GUS REPORTER*

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## Introduction

*Anthurium andraeanum* is among the most important floricultural crops in many tropical and sub-tropical countries in the world including Sri Lanka. It is one of the major sources of cut flowers trade worldwide. A huge number of novel Anthurium cultivars are required to provide floricultural industry with diverse color and shaped flowers and plants to address the increasing demand for cut flowers in the global market. Thus, breeding strategies should be adapted to address the consumer preferences. Also improving the local cultivars could cater to both local and export market providing elite cultivars. *Agrobacterium*-mediated genetic transformation is the dominant technology used for the production of transgenic plants. Recombinant *Agrobacterium* strains, in which the native T-DNA has been replaced with genes of interests, are used for efficient plant transformation. There are several concerns on transgenic plants such as horizontal gene transfer and allergies. Therefore, investigations on novel transformation methods to overcome above concerns would motivate the breeders to produce safe genotypes through genetic engineering. This study was carried out with the objective of determining the effect of seedling transformation of Anthurium using *Agrobacterium* carrying *35S: GUS* as a plant gene transformation method.

## Materials and Methods

*Plant materials, growth conditions and culture media*

Eight months old seedlings of *Anthurium andraeanum* variety *Makandura* from Sri Lanka were grown on MS medium under the short-day (SD) condition (8h light and 16h darkness).

*Agrobacterium-mediated transformation, Co-cultivation and Selection*

*Agrobacterium tumefaciens* strain LBA 4404 harboring the pBI121 vector carrying *GUS* gene under the control of the CaMV 35S promoter, and *NPTII* gene under NOS promoter were used for the transformation.

The *Agrobacterium* liquid culture for transformation was prepared using Luria broth (LB) medium (2g/100ml) with the antibiotic Kanamycin 50mg/l by inoculating a colony of the bacterial culture. The prepared liquid culture was kept overnight in a shaker incubator with mild shaking to facilitate the growth.

The transformation was carried out by immersing the entire seedlings in *Agrobacterium tumerfaciens* suspension for 20 minutes. The transformed seedlings were transferred to the co cultivation (antibiotic free medium) medium for 3 days. After the co cultivation the explants were washed with sterilized distilled water and transferred in to antibiotic containing selection medium containing MS supplemented with the antibiotic Kanamycin 50mg/l.

*GUS histochemical assay for transgene expression*

Ten putative transformed seedlings on selection medium were histochemically tested for β-glucuronidase activity after one week of transformation. The materials for staining were incubated for overnight at 37±1 $^{0}$C in *GUS* assay solution composed of X-Gluc solution [(1mM X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid), 50 mM NaH2PO4, pH 7.0 , 0.1% Triton X-100] . The seedlings were then transferred to 75% ethanol to remove the green coloration imparted by chlorophyll. A negative control of non-transformed seedlings was also assayed.

*Data collection*

The observations were recorded as the number of explants that showed a blue coloration from the *GUS* histochemical assay.

**Results and Discussion**

*Gus reporter gene expression in Anthurium seedlings*

The transformed seedlings survived on antibiotic selection medium during the short experimental period were compared to the non-transformed Anthurium seedlings. After two weeks of transformation, the putative transgenic seedlings indicated *GUS* activity as identified from the blue spots on the anthurium seedlings, whereas control seedlings were white after the assay. Above results indicated the possibility for seedling transformation for anthurium. Although Anthurium is one the most-studied plants for gene transformation, achieving highly efficient and consistent expression in Anthurium remains challenging.

Above attempt on a convenient and simple transformation method for Anthurium will open up new vista for the genetic improvement of new and targeted colours to the spathes and spadix. *Agrobacterium*-mediated transformation using β-glucuronidase (*GUS*) as a reporter is a simple, fast, reliable and robust expression system in Arabidopsis seedlings as well [2].

Concentration of bacterial cells in the induction medium is an important factor to be considered for efficient transformation. Very low density of bacterial population could lead to ineffective transformation, whereas very high density may lead to death of the explants. Also the complete elimination of bacteria from the explants after co-cultivation is crucial, as it will interfere with the growth and organogenesis of the explants. Overgrowth of bacteria causes death

of the explants and disrupts the experiment. Elimination of bacteria from the explants is carried out with the help of antibiotics [3].

**Conclusions and Recommendations**

Seedling transformation using 35S:*GUS* is successful in anthurium variety *Makandura*. Bio safety and the environmental effect of above anthurium should be confirmed by further studies.

**Acknowledgement**

We gratefully acknowledge Prof. Tomohiro Kiyosue, Kagawa University, Japan for the kind gift of pBI121 vector containing *GUS* gene.

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# INVESTIGATION OF HYDROXYAPATITE NANOPARTICLES TOXICITY ON *Raphanus sativus*

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## Introduction

Modern-day fertilization is focused mainly on preventing nutrient losses and on enhancing the availability of nutrients to crops for efficient uptake. Nanotechnology has given promising results to overcome these challenges of conventional fertilization [1]. At present, the application of nanofertilizers has different aspects, out of them the development of more efficient macronutrient nanofertilizers has given more attention than any other. Most of the researches have focused on synthesizing nitrogen (N) and phosphorus (P) nanofertilizers in order to enhance fertilizing capacity with high efficiency, low leaching rate and lower risk to the environment [2]. Therefore, higher significance has given on nanofertilizers to enhance the efficiency of nutrient use. Accordingly, Liu and Lal, [3] have reported Hydroxyapatite nanoparticles (HA NPs) as a better P nanofertilizer for modern fertilization that could enhance agricultural production. In addition, Kottegoda *et al*. [4] synthesized urea-modified HA NPs encapsulated in *Gliricidia sepium* nanocomposite for slow release of N.

However, there are concerns on the toxicity of HA NPs to the environment at higher concentrations although, it has been recommended as a P nanofertilizer. Only a single report available in the literature evident that Carboxy-methyl cellulose stabilized HA NPs had no toxic effects on *Solanum lycopersicum* L, however the range tested was 0-1000 ppm excluding its higher concentrations [1]. Therefore, it is vitally important to investigate the effect of higher concentrations of HA NPs on agricultural crops. Further, many studies have evidenced that there is a higher potential of P fertilizers to show toxic effects on crops by complexing with elements such as Fe, Mn and Zn [5]. Therefore, this study intends to determine the effect of a higher dose of HA NPs of 10,000 mg/L on *Raphanus sativus* with respect to soluble protein content.

## Materials and Methods

*Synthesis of Hydroxyapatite nanoparticles*

Synthesis of HA NPs were performed using aqueous solutions of Calcium hydroxide ($Ca(OH)_2$) and Ortho-phosphoric acid ($H_3PO_4$, 85%), both of analytical grade. Briefly, 0.6 M $H_3PO_4$ (250 mL) was added dropwise into an aqueous

suspension of $Ca(OH)_2$ (19.29 g of $Ca(OH)_2$ in 250 mL of distilled water), while stirring strenuously under mechanical agitation (1000 rpm) at room temperature. HA NPs thus synthesized as explained above were allowed to settle and the supernatant was discarded. HA NPs were washed with distilled water and dried at 100 °C to obtain dry powder.

*Characterization of HA nanoparticles*

Synthesized HA NPs were characterized with X-ray diffraction (XRD) using a Rigaku Ultima IV X-Ray Diffractometer with a CuKα radiation (1.5406 Å) over a 2θ measuring range of 3 to 60° with scan speed of 4° $min^{-1}$ at 30 mA and 40kV. Size and shape HA NPs were analyzed using Scanning electron microscopy (SEM; HITACHI SU6600 microscope).

*Plant experiments*

In a preliminary experiment, *R. sativus* seedlings were treated with concentrations of HA NPs ranging from 10-10000 mg/L and it was shown that HA NPs did not show any toxic effect on *R. sativus* up to 10000 mg/L with respect to shoot biomass. An inert growing medium comprising of coir was used to avoid the possible interferences from the complicated soil components. Three *R. sativus* seeds were sowed in each plastic plant pots filled with coir. Fertilizing treatments were used in this study included Hoagland solution without HA NPs and Hoagland solutions with 10000 mg/L of HA NPs. Fertilizer solutions were applied weekly to each container at 0.25 L solution per pot. The fertilizing started when the germinated *R. sativus* were about 10 days old and stopped when plants were 45 days old.

*Electron microscopic analysis of HA NPs treated plants*

Roots of 10000 mg/LHA NPs treated *R. sativus* were taken and samples were air dried for 2 days. The roots samples were analyzed using SEM and Energy Dispersive X-ray Spectroscopy (EDX)

*Determination of soluble protein content*

*R. sativus* leaves of same position were obtained and 100 mg of fresh leaf samples were homogenized with 1.0 mL of 50 mM phosphate buffer (pH 7.4) and centrifuged at 9000 rpm for 15 min. Then, 100 µL of supernatant was mixed with 5 mL of Bradford reagent and absorbance was measured at 595 nm.

*Statistical analysis*

All data were statistically analyzed by two sample t test using Minitab 17.1. Treatment differences were assessed at $p < 0.05$.

## Results and Discussion

*Characterization of HA nanoparticles*

The XRD pattern (Figure 5) of HA NPs synthesized are in accord with previously reported XRD files for HA NPs. SEM images of HA NPs exhibited rod-like morphology with an average diameter of 20 nm and an average length of 150 nm.

*Electron microscopic analysis of HA NPs treated plants*

SEM imaging did not clearly show the presence of HA NPs on *R. sativus* root samples, but the EDX analysis confirms the successful internalization HA NPs showing that NPs might have been undergone dissolution and biotransformation within *R. sativus.*

*Determination of soluble protein content*

Soluble protein content is an important physiological parameter in the development of plant growth. Statistical results show no significant difference between 10000 mg/L HA NPs and 0 mg/L concentrations (figure 1, $p > 0.05$). The results therefore revealed that HA NPs does not induce modifications to the protein levels in plants during their growth.

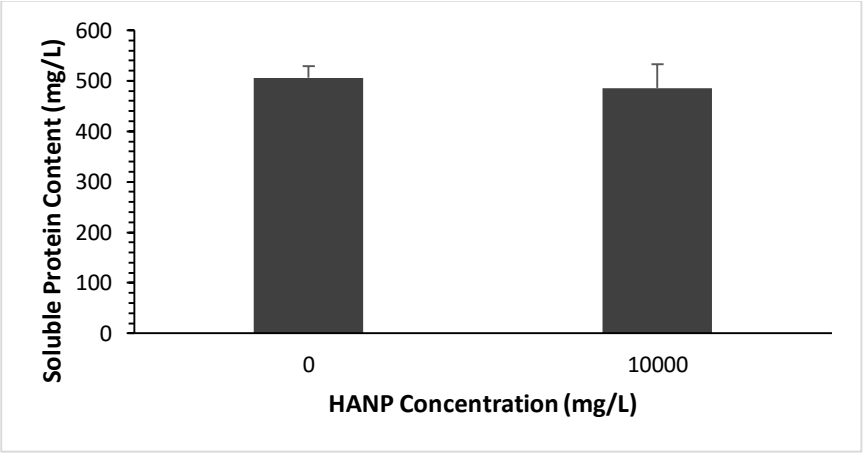
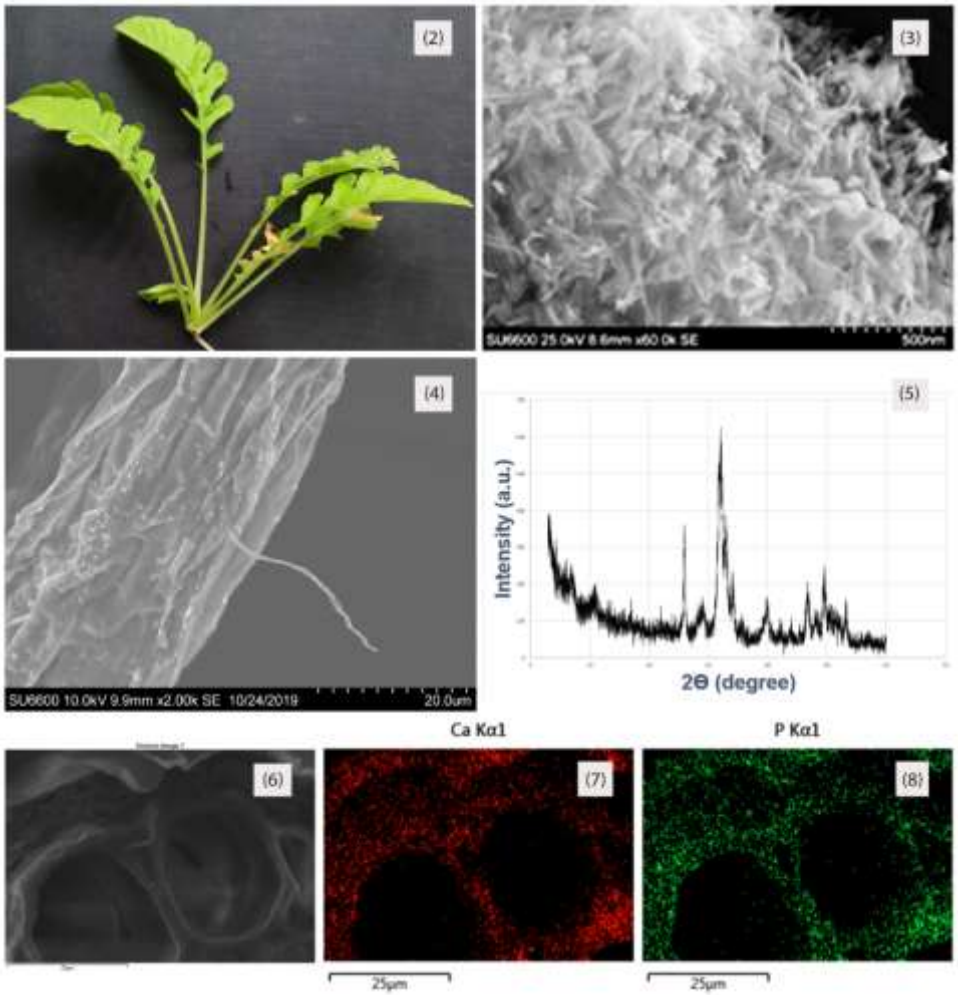



**Figure 1.** The leaf soluble protein content (mean ±SD, n=3) of 45 days old *R. sativus* treated with 10000 mg/L HANPs.

Many of the studies on phytotoxicity of nanomaterials to model plants were tested under hydroponic systems but this study was conducted under inert solid support (coir fiber) to provide more-closer conditions to soil systems.




**Figures 2.** *R. sativus* (3) Scanning electron microscope images of synthesized HA nanoparticles (4) Scanning electron microscope image of a root of HA NPs treated *R. sativus* root (5) XRD pattern of synthesized HA NPs (6, 7, 8) EDX results of *R. sativus* root

## Conclusion

Nanotechnology has provided the license to utilize different nanoparticles in agriculture as effective fertilizing agents. Present study demonstrates that bare-HA NPs has no toxic effects on *R. sativus* under plant growth conditions provided. Therefore, this study validates previous publications on HA NPs as an effective P supplier for agricultural crops. Furthermore, this study will concern the fate of nanomaterials under solid medium which resembles a typical agricultural field.

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# EVALUATING THE EFFECTIVENESS OF A VIDEO-DOCUMENTARY AS A TOOL TO PROMOTE CLIMATE SMART AGRICULTURAL PRACTICES AMONG FARMING COMMUNITIES IN SRI LANKA

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## Introduction

Sri Lanka is a vulnerable island from climate changes [1]. As a result of climate change, the yield of paddy, vegetable, other food crops have declined considerably over the years. Paddy production during Yala (2015) and Maha (2016) seasons reduced by 46.1 % due to adverse effects of climate. The agriculture sector contributes about 6.9% to the national Gross Domestic Production. Over 27.1% of Sri Lankans are employed in the agriculture sector [2]. Any adverse changes in the sector can harm the economy of Sri Lanka because the economy is significantly agrarian and the majority of the rural community is depending on Agriculture [3]. Therefore, adapting to Climate-Smart Agricultural (CSA) practices has been identified as one of the strategies to minimize the adverse effects of climate change on agriculture. Since the CSA concept is new to Sri Lanka there should be a necessary dissemination approaches to promote and motivate farmers towards CSA. There are great potential and imperative demand for promoting the CSA to be chosen as the solution among farmers [4]. Mass media can reach effectively and efficiently to people rather than teaching something because of the attractive features, retention ability and interest audio-visual media have[5]. Hence the study aimed at producing a video document to motivate farmers towards CSA practices through motivational and attitudinal changes and evaluate the effectiveness.

## Materials and Methods

The study was conducted as an action research and the production process consisted of three main phases.

*Process for video production*

**Table 1.** Video production phases and steps

| Production phase | Steps |
|---|---|
| Pre-production | • Pre-survey on the awareness about CSA practices<br>• Identify the treatment dimensions<br>• Concept and Framework design<br>• Visual script and background sound and music selection<br>• Location, set, crew and costumes selection |
| Production | • Recording audio and video |
| Post-production | • Video editing<br>• Pre-testing<br>• Post-testing / evaluation test<br>• Replication |

First a pre-survey was conducted as key personal interviews with twenty respondents in two selected communities namely Sivalakulama and Bandarakunukwewa cascades, North central province, Sri Lanka. During the survey knowledge and attitude gap, awareness about CSA practices among farmers and their preferred mode of information receiving methods were identified.

Based on the findings of the pre-test a script was developed and field audio and videos recordings were done using professional equipment. Before testing the video with farmers, a pre-test was conducted with 35 graduate students to assess the effectiveness of the video to disseminate information to farming communities using following variables visual attractiveness, visual appropriateness, understandability, dialogue attractiveness, visual graphics quality, audio clarity and the capability of motivating farmers towards CSA.Then the video was tested with randomly selected 60 farmers (Age 18-35) from Sivalakulama and Bandarakubukwewa cascade to measure the videos' effectiveness among farming communities and to test the change in attitude and motivation to practice CSA before and after watching the video. A structured questionnaire was used to collect responses.

**Results and Discussion**

In the pre-survey, 50% of the respondents believed climate change is the main reason for the loss of their yield and 10% didn't have an idea about climate changes. Half of the respondents (50%) preferred to receive knowledge on CSA in the format of a docudrama.

At the pre-test majority of graduates strongly agreed that the video was attractive (71%), understandable (77%), of good visual quality (63%),

appropriately disseminate knowledge (89%), motivate framers towards CSA (74%) and of good overall quality (86%) (Table 2).

To evaluate the effectiveness of the video among farming communities a post-test was conducted with 60 rural farmers. The attitude and knowledge change before and after watching the video were tested using the paired t-test. Attitude and knowledge change about CAS were highly significant (*p<0.05*) (Table 3). After watching the video about 91% of the respondents were willing to practice CSA in the future (perceived willingness to practice). To ensure understandability of the video a pooled t-test was conducted between farmer's and graduate's responds. Understandability and attractiveness of visuals and dialogues were not significantly different (*p>0.05*) (Table 4).

**Table 2.** Result of pre-test with graduates

| Variable | Strongly Agree % | Partially Agree % | Neutral % | Partially Disagree % | Strongly Disagree % | Mean Rank |
|---|---|---|---|---|---|---|
| **Rank** | **5** | **4** | **3** | **2** | **1** | |
| Visual Attractive | 71 | 29 | 0 | 0 | 0 | 4.71 |
| Visual appropriate | 80 | 20 | 0 | 0 | 0 | 4.8 |
| Visual Understandable | 77 | 20 | 3 | 0 | 0 | 4.74 |
| Dialogue attractive | 49 | 46 | 3 | 3 | 0 | 4.4 |
| Dialogue appropriate | 69 | 29 | 3 | 0 | 0 | 4.65 |
| Visual Understandable | 83 | 17 | 0 | 0 | 0 | 4.82 |
| Visual Graphic Quality | 63 | 34 | 3 | 0 | 0 | 4.6 |
| Audio Clarity | 57 | 34 | 3 | 6 | 0 | 4.42 |
| Overall concept is good | 86 | 11 | 3 | 0 | 0 | 4.82 |
| Capable of Giving CSA idea | 89 | 11 | 0 | 0 | 0 | 4.88 |
| Capable of motivate farmers to CSA | 74 | 26 | 0 | 0 | 0 | 4.74 |

**Table 3.** Farmers attitude before and after watching the video (paired t-test)

| Variable | Mean Rank Before | Mean Rank After | P-value |
|---|---|---|---|
| Framers can face climate change successfully | 1.2 | 4.45 | 0.000 |
| Agriculture gives a good income | 2.45 | 4.17 | 0.000 |

*Significantly different at p < 0.05*

**Table 4** Comparison of the response of graduates and rural farmers (pooled t-test)

| Variable | Mean Rank by Graduates | Mean Rank by Framers |
|---|---|---|
| Visual Attractive | 4.71 | 4.42 |
| Visual Understandable | 4.74 | 4.48 |
| Dialogue attractive | 4.4 | 4.05 |
| Dialogue Understandable | 4.82 | 4.71 |
| Capable of Giving CSA idea | 4.88 | |
| Capable of motivating farmers to CSA | 4.74 | |
| Farmers can face Climate changes successfully | | 4.45 |
| Climate-smart agriculture will be given a good income | | 4.17 |
| Willingness to practice CSA | | 4.88 |

**Conclusions and Recommendations**

The dry zone agricultural communities don't have a clear idea about climate change. Their awareness about CSA practices is very poor. Video documentary is an effective tool to make farmers aware and motivate them towards CSA practices. This produced video could be used as a training tool to make farmers aware about adverse effects of climate change and to motivate them to adopt CSA practices. Translations of this video in Tamil and English and making them available in public and social media is recommended for future actions.

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# ALLELOPATHIC EFFECTS OF SELECTED PLANT EXTRACTS ON THE GROWTH OF YELLOW NUTSEDGE (*Cyperus esculentus* L.)

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## Introduction

Yellow nutsedge (*Cyperus esculentus*) is a perennial weed which is widespread in tropics and sub tropics. It is a major weed in paddy fields in Sri Lanka. Allelopathy is identified as a natural weed control approach. Allelopathy is one plant's directly affecting another plant's growth either positively or negatively, exuding chemical substances. Several plant species posses such ability to release allelopatic compounds. These compounds are found in different plant parts viz. root, stem, rhizomes, flowers and leaves. However, nature of the allelopathic potential of most of the species has not been identified yet. *Nerium oleander* and *Thevetia peruviana* are evergreen shrubs considered with some allelopathic potential. Al-Samarai *et al*., [1] reported that, aqueous extract of *N. oleander* leaves showed inhibitory effects on germination and seedling growth of purple nut sedge. Pavithra *et al*. [2] reported the suppressive effect of *T. peruviana* on *Parthneium hysterophorus*. Hence, alleopathic potential of *N. oleander* and *T. peruviana* could be utilized to control major weeds of Sri Lanka in an environment friendly manner. Hence the present study was conducted to evaluate the effect of different forms and concentrations of *N. oleander* and *T. peruviana* leaf extracts on the growth of yellow nutsedge.

## Materials and methods

*Duration and location*

This experiment was conducted from January to April 2019 at the Crop Science Laboratory, Faculty of Agriculture, Eastern University, Sri Lanka.

*Experimental design and treatments*

The experimental design was Completely Randomized Design (CRD) with fourteen Treatments. Each treatment was replicated three times. Following treatments were imposed: T1(Control distilled water), T2 (100% coconut Vinegar) , T3(100% *Nerium oleander* dry leaf extract) ,T4(75% *N. oleander* dry leaf extract), T5(50% *N. oleander* dry leaf extract), T6 (100% *N. oleander* fresh leaves extract), T7( 75% *N. oleander* fresh leaves extract),T8 (50% *N. oleander* fresh leaf extract),T9 (100% *Thevetia peruvian* dry leaf extract), T10 (75% *T. peruvian* dry leaf extract ), T11 (50% *T. peruvian* dry leaf extract), T12(100% *T.*

*peruvian* fresh leaf extract), T13 (75% *T. peruvian* fresh leaf extract) and T14 (50%*T. peruvian fresh* leaf extract).

*Preparation of dry leaf extracts*

Fresh leaves of *T. peruviana* and *N. oleander* were collected, cleaned and dried under shade for one week. Dried leaves were ground into a fine powder and stored in airtight containers. Extracts of the leaves were prepared by dipping 50 g of air dried leaves powder in 100 ml vinegar for 24 h at 25 ± 5°C. Extract was filtered through Whatman filter paper (No.1) and the volume of filtrate made to 100 ml. Different dilutions of the extracts i.e. 100%, 75%, and 50% were prepared from stock solution by adding distilled water.

*Preparation of wet leaf extracts*

Collected fresh leaves were cleaned and ground into a paste form. Then extracts of the leaves were prepared by dipping 50 g of leaf paste in 100 ml vinegar for 24 hr at 25 ± 5°C. Extract was filtered through Whatman filter paper (No.1) and the volume of filtrate made to 100 ml. Different dilutions of the extracts i.e. 100%, 75%, and 50% were prepared from stock solution by adding distilled water. The resulted extracts were stored under ambient condition in the laboratory.

*Preparation of pot*

Potting media was prepared by mixing compost and sand at a ratio of 1:2. Plastic pots were filled with the prepared potting media and used for planting.

*Planting materials*

Yellow nut sedge seedlings (two to three weeks old) were collected from paddy fields of Batticaloa district and those were planted in already prepared pots and were kept for 14 days under appropriate management conditions.

*Application of plant extract*

Leaf extracts were sprayed to foliage (2ml per plant) of the seedlings at two days interval as per treatment structure for four times.

*Measurement and data analysis*

Number of plants died was counted at fourteen days after application of first treatments. Data were analyzed with Friedman's non-parametric analysis of variance.

**Results and Discussion**

Vinegar extracts of *T. peruviana* L. and *N. oleander* L. showed significant ($p<0.05$) allelopathic suppressive effect on yellow nutsedge. Highest number (100%) of plants died was found in T9. However, no plants died in T1 and T2 (Fig. 1).

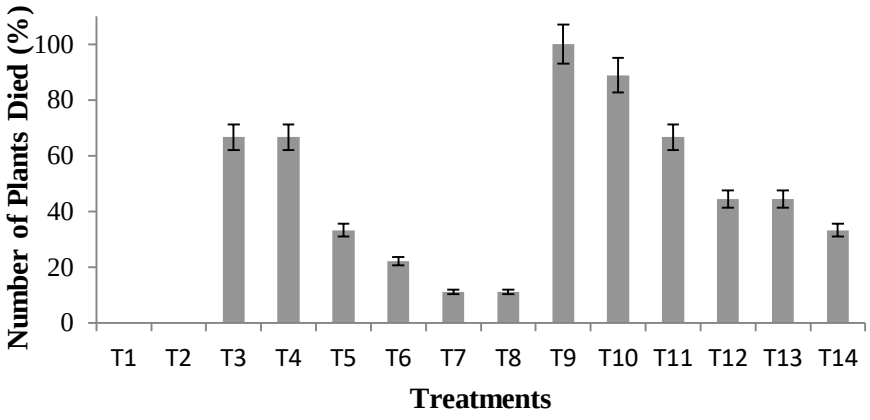


**Figure 1.** Effect of leaf extracts of *T. peruviana* L. and *N. oleander* L. on Yellow nut sedge (*Cyperus esculentus*) ($p<0.05$)

There was no significant difference between T1 (Control) and T2 (Vinegar) and no plants were died in these treatments. It was reported that acetic acid (vinegar) has some herbicidal effect when applied at higher concentration.

However it was found that, vinegar has no suppressive effect on yellow nutsedge. Smith-Fiola and Gill [3] pointed out that; vinegar is effective against very small annual broadleaf weeds through affecting the cell membranes of a plant, causing rapid breakdown desiccation of foliage tissue on contact [3]. Perennial nature could be a potential reason for non-suppressive effect of vinegar on yellow nutsedge.

Deaths of yellow nutsedge were recorded in Treatments 3 to 14. Therefore vinegar leaf extracts of *T. peruviana* and *N. oleander* had allelopathic effect on yellow nutsedge in different forms (wet and dry) and concentrations (50, 75 and 100%). Arora [4] reported about alleopathic potential of *T. peruviana* on *Triticum aestivum* L. seed germination. Uslu *et al*. [5] reported the allelopathic effects of *N. oleander* flower extract on the germination and seedling growth of *Lolium multiflorum*.

The inhibitory potential of vinegar leaf extracts of *T. peruviana* and *N. oleander* increased with increasing extract concentrations of extract in wet and dry forms. It was also observed that dry leaf extracts of *N. oleander* (T3, T4 and T5) and *T. peruviana* (T9, T10 and T11) had potent allelopathic activity than wet forms of respective leaf extract. Dry plant materials facilitate free bonding of solvents while wet samples produce viscosity and lack of separation of secondary metabolites corresponding to polarity of solvent used. Further there were

reported cases where, dried leaves were used for the extraction of allelochemicals from *T. peruviana* [4] and *N. oleander* [5].

Significantly ($p<0.05$) highest number of death plants were recorded in T9. Number of dead plants was higher in *T. peruviana* extract applied treatments than *N. oleander* treatments. It showed that *T. peruviana* leaf extract had higher allelopathic suppressive effect on yellow nutsedge than *N. oleander*.

**Conclusions and Recommendations**

Yellow nutsedge (*Cyperus esculentus* L.) plant growth was significantly affected by vinegar leaf extracts of *Thevetia peruviana* and *Nerium oleander.* The inhibitory potential of vinegar leaf extracts of *T. peruviana* and *N. oleander* species increased with increasing concentrations of extract in wet and dry forms. Dry leaf extracts of *N. oleander* and *T. peruviana* exhibit potent allelopathic activity than wet forms of respective leaf extracts. Leaf extract of *T. peruviana* has higher allelopathic suppressive effect on yellow nutsedge than *N. oleander*. Vinegar leaf extracts of *T. peruviana* and *N. oleander* could be used to control yellow nutdegde in an environment friendly manner. However further studies are needed before making a firm recommendation.

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# SELECTED PLANT PATHOGENIC FUNGAL CULTURE FILTRATES (SECONDARY METABOLITES) AS POTENTIAL BIO-HERBICIDES

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## Introduction

A weed is considered unwanted plant in a particular situation when it grows in a wrong place. Weeds remain problematic to agriculture, forestry, rural and urban landscaping, transport especially motorways and railway tracks, and fresh water ecosystem except their use in indigenous medicine as medicinal herbs. Weeds can be controlled by manually, mechanically, biologically and chemically. The discovery and introduction of the phenoxyacetic herbicides laid a novel era in the field of agricultural weed control. World consumption of herbicides is 44 % [1].

Herbicides are synthetic chemicals that cause many health issues to human, wild and farm animals. Moreover, application of such toxic chemicals not only may affect ecosystem functions such as productivity and food web interactions, and thus the services aquatic ecosystems provide [2], but also may cause unintentional injury to crop and other non-target vegetation and other organisms in an area by faulty application techniques. Consumers are not willing to by herbicides applied fruits and vegetables. Herbicide usage has resulted in plant evolution and adaptation by the selection of genetic traits conferring phenotypic resistance and allowing weedy plants to survive and reproduce in the presence of herbicides. Weeds have evolved resistance to 23 of the 26 known herbicide sites of action and to 163 different herbicides. Due to the hazardous nature of herbicides (Glyphosate, Atrazine, Metam and etc.), many herbicides have been banned in worldwide. The complexity of these situations has resulted in a need to develop a holistic sustainable eco-friendly weed management programme throughout the farming period. Ecofriendly secondary metabolites like ethylene-inducing peptide 1–like (NLP) proteins from microbes are now being targeted to produce novel herbicides and several successful findings have been reported in recent literatures.

*Phytophthora infestans* is the causal agent for late blight in potato and *P. infestans* producing NLPs and their mode of action have been identified [3]. *Botrytis cinerea* is also one of the Ascomycete fungi causing disease on crops called gray mold. *Botrytis cinerea* produces a range of cell-wall-degrading enzymes, toxins and other low-molecular-weight compounds such as oxalic acid [4]. *Fusarium solani* is implicated in plant disease as well as human disease. This

species can decompose cellulose at an optimal pH of 6.5 and temperature of 30°C. Culture filtrates of *Fusarium solani* can be used to control weeds [5].

According to above information, this investigation was planned to study the potential of using the microbial toxins of *Phytophthora infestans, Fusarium solani* and *Botrytis cinerea* as an effective bio herbicide to control weeds.

## Materials and Methods

*Sample collection and Isolation of test fungi species*

All the disease samples were collected (Research Field) and Isolation of *Phytophthora infestans*, *Fusarium Solani*, *Botrytis cinerea* were carried out at the Agriculture Research and Development Centre (National Potato Research Centre) Seetha Eliya, Nuwara Eliya, Sri Lanka located in 7°0'31"N 80°47'39"E.

*Preparation of test fungi culture filtrates*

Metabolites of *P. infestans* were prepared by inoculating a fungal agar plugs (1 $cm^2$) from the periphery of the 21 days old culture to pre-autoclaved Pea broth in 250 ml flasks under aseptic conditions. Conical flasks of PDB (Potato Dextrose Broth) were separately inoculated by one-week old *F. solani* culture and two weeks old *B. cinerea* cultures. Each flask was inoculated with the 1 $cm^2$ size agar plugs which were taken from the periphery of the fungal culture plates. The fungus *P. infestans* inoculated flasks were kept in the water bath shaker at 20 °C in 150 rpm for one month in the dark condition. *B. cinerea* and *F. solani* inoculated flasks were kept in Orbital shaker at the room temperature (18 °C) in 150 rpm for twenty-one days (twelve ours light and twelve hours dark condition). The liquid cultures of filtered through the sterilized nitrocellulose membrane filters of 45 µm pore size by using vacuum condition.

*Screening the effect of microbial toxin on selected weeds*

Pots of 5 Kg capacity were prepared by using black polythene. Each pot was filled with standard sterilized potting mixture. Matured seeds of most problematic weed species such as *Asclepias verticillata*, *Rorippa micrantha*, *Panicum repens* and *Cyperus rotundus* were collected and dried in the normal room temperature for a week. Dried seeds were planted in pots as four seeds per pot and watered once in two days to maintain at field capacity. Only one species was planted in one pot. Each species was replicated four times for each treatment. Pots were kept under the natural environment. 400 mL of each treatment was prepared (Table 1) and sprayed equally to each replicates of every weed species. Hence, 25 ml of treatment was sprayed on each pot. Glyphosate (isopropylamine salt) 360 g/l SL was used for the treatment preparation. Glyphosate solution was made according to the standard {100-125 ml of Glyphosate should be dissolved in 10 L of water} (DOA, 2009). Treatments was applied in each pot using hand sprayer in an early morning of 6 a.m.

**Table 1.** Treatments for the pot experiment

| Treatment Number | Treatment Materials |
|---|---|
| A-Treatment 1 | Distilled water (Control) |
| B-Treatment 2 | Glyphosate (Standard) |
| C-Treatment 3 | *Fusarium solani* culture filtrate |
| D-Treatment 4 | *Phytophthora infestans* culture filtrate |
| E-Treatment 5 | *Botrytis cinerea* culture filtrate |
| F-Treatment 6 | Mixture of *P. infestans + F. solani + B. cinerea* culture filtrate |

*Scoring of leaf yellowing*

Before the treatment application, foliage of each plant was 100 % green color. After the treatment application, compared to the plants in the control, the yellow color portion of the foliage of each plant and the integrity of the yellow color of the foliage under each treatment were recorded as a percentage of yellowing from 0 % to 100 % using color chart (Fig. 1)


**Figure 1.** Visual scale scoring for weeds yellowing

*Data collection and statistical analysis*

Data of shoot and root growth and percentage of yellowing of part or whole plants by visual scoring were collected. Complete randomized design (CRD) was used to perform analysis of variance (ANOVA) in SAS software version 9.4. Duncan's least significant differences (LSD) test among the treatments were calculated to show the best treatment using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

**Results and Discussion**

Secondary metabolites of different fungus on different weed species was significantly ($P$ <0.05) varied compared to control. Application of mixture of secondary metabolites (Treatment 6) exhibited tremendous impact and highly significant on weed with the yellowing percentage of 80 % and 60 % on *R. micrantha* (Fig. 3) and *A. verticillata* respectively (Fig. 4). But individually, *F. solani* culture filtrate has given a considerable yellowing effect 40 % and 30 % on same species respectively than other weed species. None of the fungal secondary metabolites as mixture or individually was not significant on *C. rotundus* and the *P. repens* except synthetic chemical Glyphosate. Treatment 2 (Glyphosate) has reached its maximum 100% effect for the broad leaves (Species 1 and Species 2) within 7 days from the application and has taken 14 days to reach about 90% effect for the Sedge (Species 3) and the Grass (Species 4). From this experiment, it is concludable that the amount of application of synthetic nasty herbicide could be reduced if applied by mixing of secondary fungal metabolites as ratio wise fraction. Further experiment is needed to confirm the results.

The mode of action of different secondary metabolites varied. The sensitivity of plants to fungal phytotoxins in the culture filtrate can be assessed by morphological alterations and inhibition of weight increase [6]. The reduced of relative chlorophyll content (SPAD) can due to toxins translocate to the aerial parts. Agrios [7] stated that the toxins produced by pathogenic fungi inhibit enzymes that are directly or indirectly involved in photosynthesis. This inhibition causes chlorophyll degradation and/or the reduction of chlorophyll synthesis, resulting in the development of leaf chlorosis. Selected dicot weeds (Species 1 and Species 2) had a yellowing effect due to the *F. solani* toxin that cause for the chlorosis in leaves.

During growth of *P. infestans*, several hydrolytic enzymes which are interfering with various plant physiological functions including cell wall-degrading activity. Therefore, mixture of the culture filtrates may have broad spectrum (interfering more than one physiological function at a time) of phytotoxic compounds from *Phytophthora infestans*, *Fusarium solani* and *Botrytis cinerea*. So that the plant leaves may get higher chlorosis than the other culture filtrates when applied individually

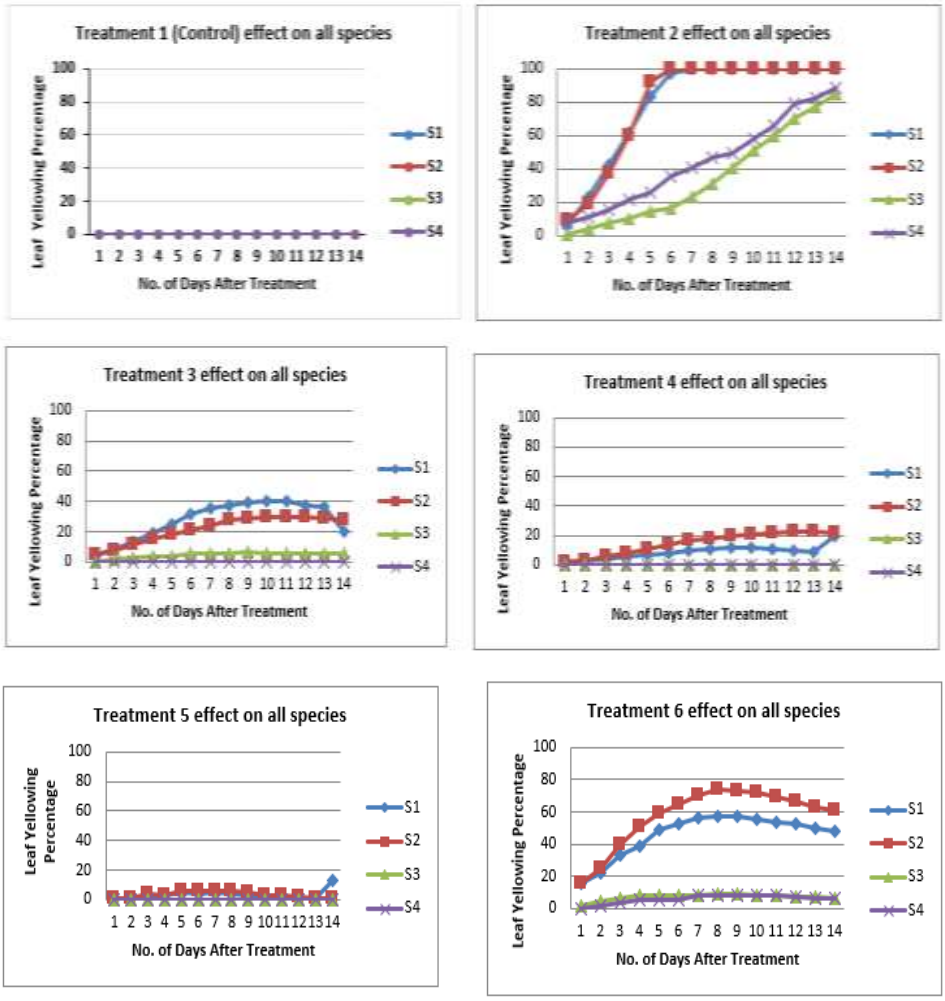


**Figure 2.** Yellowing effect of fungal culture filtrates on the weeds


Figure 3: *Rorippa micrantha* response to the fungal secondary metabolites (A) control (T1), B-*Fusarium solani* (T3), C- *Phytophthora infestaans* (T4), D- *Botrytis cinerea* and E- Mixture of culture filtrates (T6) after 7 days




Figure 4: *Asclepias verticillata* response to the fungal secondary metabolites (A) control (T1), B-*Fusarium solani* (T3), C- *Phytophthora infestaans* (T4), D- *Botrytis cinerea* and E- Mixture of culture filtrates (T6) after 7 days

**Conclusion**

In the application of mixture of the cultural filtrates gave the highest yellowing response on all the selected weed species. From this experiment, it is concludable that the amount of application of synthetic nasty herbicide could be reduced if applied by mixing of secondary fungal metabolites and synthetic chemical as ratio wise fraction. Further experiment is needed to confirm the results.

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# AN INNOVATIVE LIQUID FERTILIZER TO PROMOTE GROWTH AND YIELD RESPONSES OF TOMATO (*Lycopersicon esculentum* Mill.)

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## Introduction

Any development in agricultural system which results in higher production should also minimize the negative effects on environment. Nowadays, the excess use of inorganic fertilizer is a serious environmental issue in Sri Lanka as well as in other parts of the world. Few inorganic fertilizers are hazardous to human due to bio-magnification of toxic elements from fertilizers which accumulate into the plant and finally reach to our body and also affect the micro-organisms living in the soil. Available nutrients from inorganic fertilizers easily leach away into ground water without fully benefiting the plant and causes environmental pollution.

Organic fertilizer is an alternative source for synthetic fertilizer which is an environmentally sound product as well as an economically viable option. Organic fertilizers improve the structure, fertility and health of the soil [1]. They can produce quality agricultural products with high nutritional value. Organic fertilizers also minimize the use of non-renewable sources and increases biodiversity. Foliar application of fertilizers is one of the techniques in organic farming. It can feed the plants by applying liquid fertilizer directly to their leaves. Plants are able to absorb essential nutrients rapidly through their leaves therefore it is the best method when prompt correction of nutrient deficiencies is required and also during adverse condition such as drought and, disease and pest attacks.

On this background a field experiment was conducted at Regional Agricultural Research and Development Centre (RARDC), Kilinochchi to study the effects of different organic foliar applications, namely seaweed extract and *Azolla* formulation (azolla extract + cow dung + cow urine) with combination of organic and inorganic recommendation of DOA using *Maheshi* variety of *Lycopersicon esculentum* Mill (Tomato) as the test crop.

## Materials and Methods

*Preparation of Azolla formulation (Azolla filiculoides)*

*Azolla* formulation was prepared by using azolla extract and decomposed solution. *Azolla* used in this study was collected from organic unit of RARDC. It was washed with tap water to remove unwanted impurities. Fresh azolla samples were blended without adding water and strained through a white cloth to get *azolla* Extract. It was preserved in refrigerator.

10 Kg of Fresh cow dung, 500 ml of cow urine and 10 L 0f water were taken with the ratio of 20: 1: 20 to prepare decomposed solution. Mixture of contents was allowed to decompose for about 2 weeks. Decomposed organic liquid fertilizer solution was filtered by using a white cloth and preserved in refrigerator. For the preparation of *Azolla* formulation, 650 ml of *Azolla* extract, 2 L of decomposed solution and 7.35 L of water were taken into a small container and it was mixed thoroughly to prepare 10 L of azolla formulation. After 30 minutes, *Azolla* formulation was ready to use.

*Preparation of seaweed extract (Kappaphycus alvarezii)*

The red algae were handpicked from the coastal area of vallaipadu during December 2018. It was transported to RARDC, Killinochchi and washed with tap water to remove unwanted impurities. Then it was blended, filtered and preserved in the refrigerator. The liquid filtrate was taken as 100% concentration and diluted as per treatment. In this study 5% of seaweed extract was used [2].

*Field experiment*

The experiment was conducted at RARDC, Kilinochchi, during *Maha* season of 2018. Study was laid out in a Randomized Complete Block Design (RCBD) with 5 Treatments namely DR, 50% DR+AZ, 50% DR+SW, 50% ORG+AZ, 50% ORG+SW. (DR: Departmental recommendation, SW: seaweed extract, AZ: *Azolla* formulation, ORG: Organic manure: Cattle manure) with 3 replicates. Seaweed extract (SW) and *Azolla* formulation (AZ) were used in this study from 2 weeks after transplanting (35 days old plants). Five foliar applications were done during the experiment in 2 weeks intervals. Foliar sprays were carried out in morning at the rate of 250 L $ha^{-1}$. All other management practices were done as per the recommendation of department of agriculture. Data were collected on plant height, leaf number per plant and fruit yield.

*Statistical analysis*

ANOVA was done using SAS (9.1) package and the mean separation was done by Duncan multiple range test at P=0.05.

## Results and Discussion

*Effects of foliar sprays on growth of tomato*

The plant height at 2 weeks interval is illustrated in Table 1. The highest plant height was observed in 50% DR+AZ which was statistically significant than other treatments except DR. The lowest value was noticed in 50% ORG+SW at two weeks after planting. At 4th and 6th weeks after planting highest and lowest plant height was recorded with DR and 50% ORG+SW respectively. At 8 weeks after planting highest height was observed in 50% DR+AZ and it has significant effect with organic treatments. There is no significant difference among the treatments namely DR, 50% DR+AZ and 50% DR+SW.

**Table 1.** Effects of foliar sprays on tomato plant height and leaf number

| | 2WAP | | 4WAP | | 6WAP | | 8WAP | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **PH** | **LN** | **PH** | **LN** | **PH** | **LN** | **PH** | **LN** |
| DR | $12.23^{ab}$ | $6^{a}$ | $24.96^{a}$ | $12^{a}$ | $55.49^{a}$ | $31^{a}$ | $73.01^{a}$ | $49^{a}$ |
| 50%DR+AZ | $13.16^{a}$ | $7^{a}$ | $23.93^{ab}$ | $11^{a}$ | $55.40^{a}$ | $29^{a}$ | $80.30^{a}$ | $54^{a}$ |
| 50%DR+SW | $11.16^{bc}$ | $6^{a}$ | $22.23^{ab}$ | $9^{ab}$ | $46.73^{ab}$ | $21^{a}$ | $66.24^{ab}$ | $39^{ab}$ |
| 50%ORG+AZ | $10.74^{bc}$ | $5^{b}$ | $18.43^{bc}$ | $8^{b}$ | $36.29^{b}$ | $14^{b}$ | $52.63^{b}$ | $24^{b}$ |
| 50%ORG+SW | $09.90^{c}$ | $5^{b}$ | $15.00^{c}$ | $7^{b}$ | $30.84^{b}$ | $12^{b}$ | $52.00^{b}$ | $23^{b}$ |

Same letters within columns are not statistically different at p=0.05. WAP: weeks after planting, PH: Plant height in cm, LN: leaf number per plant.

Number of leaves per plant at 2 weeks intervals until 8WAP is shown in Table 1. There was no significant difference either within organic treatments or inorganic treatments throughout the growing season. Highest and lowest leaf number was recorded with 50% DR+AZ and 50% ORG+SW respectively. Our findings coincide with those of earlier studies carried out on *Pisum sativum* with *azolla* extract [3] where *azolla* extract promoted the crop growth in terms of seedling length and root length.

*Effect of foliar sprays on yield of tomato*

Fruit yield was recorded weekly. Fruit yield of each treatment at different time interval is illustrated in Table 2. The yield of first week of harvest ranged between 0.44 T $ha^{-1}$ (50% ORG+SW) to 3.44 T $ha^{-1}$ (50% DR+AZ). There was no significant difference neither within inorganic alone and combination (DR, 50% DR+AZ and 50% DR+SW) nor organic combination treatments (50% ORG+AZ and 50% ORG+SW). However significant difference was observed between organic and inorganic treatments.

**Table 2.** Effects of foliar sprays on tomato yield.

| | YIELD (T ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **1st Week** | **2nd Week** | **3rd Week** | **4th Week** | **5th Week** | **Total** |
| DR | 2.97$^{a}$ | 2.80$^{bc}$ | 4.71$^{c}$ | 3.33$^{a}$ | 2.18$^{ab}$ | 15.99$^{b}$ |
| 50%DR+AZ | 3.44$^{a}$ | 4.75$^{a}$ | 13.05$^{a}$ | 3.74$^{a}$ | 2.78$^{a}$ | 27.76$^{a}$ |
| 50%DR+SW | 2.42$^{a}$ | 3.62$^{ab}$ | 8.43$^{ab}$ | 3.10$^{a}$ | 1.69$^{bc}$ | 19.25$^{b}$ |
| 50%ORG+AZ | 0.67$^{b}$ | 2.42$^{c}$ | 8.27$^{b}$ | 4.35$^{a}$ | 1.26$^{c}$ | 16.96$^{b}$ |
| 50%ORG+SW | 0.44$^{b}$ | 2.07$^{c}$ | 7.99$^{b}$ | 4.36$^{a}$ | 1.88$^{bc}$ | 16.74$^{b}$ |

Same letters with in columns are not statistically different at p=0.05

At 2nd week of harvest, 50% DR+AZ had the highest yield (4.75 T ha$^{-1}$) compared to all other treatments which is statistically significant among all treatments except 50% DR+SW. At 3rd week of harvest, the highest and lowest yield were obtained from 50% DR+AZ and 50% ORG+SW respectively. There was no significant difference among organic treatments. 50% DR+AZ treatment had significantly higher yield at 3rd week of harvest compared to all other treatments except 50% DR+SW. At 3rd week of harvest, organic treatments had better yield than DR. At 4th week of harvest, there was no significant difference in yield among all the treatments. Yield ranged from 3.1 T ha$^{-1}$ (50% DR+SW) to 4.5 T ha$^{-1}$ (50% ORG+AZ, and (50% ORG+SW). At 4th week of harvest 50% ORG+AZ, and 50% ORG+SW reached the yield level of other treatments (50% DR+SW, 50% DR+AZ, DR). At 5th week of harvest, the highest yield and lowest yield were obtained from 50% DR+AZ and 50% ORG+AZ respectively. Moreover 50% DR+AZ had significantly higher yield than that of all other treatments except DR.

In the case of cumulative yield up to 5th week of harvest, the highest yield (27.761 t ha$^{-1}$) was obtained from 50% DR+AZ and it was significantly higher compared to all other treatments. However, there was no significant difference in yields among other treatments.

Earlier studies have reported that the *Azolla* Bio-fertilizer, when partially substituted for synthetic fertilizer, N produced higher rice yield and it also provides a financially attractive approach for farmers to substantially improve nitrogen use efficiency and rice yield while simultaneously reducing N loss [4]. Another study also reported that, *Azolla* gave highest biomass production and relative growth rate and it had high nitrogenase activity [5].

**Conclusions and Recommendations**

This study has revealed that, 50% DR+AZ treated plots gave significant growth and yield (27.76 T ha$^{-1}$) compared to other treatments including department recommendation. It is significant to note that by substituting 50% of department recommendation with *Azolla* formulation yield of tomato was

increased by 82% than that of DR. This study was conducted only at Kilinochchi District during *Maha* season. Therefore, experiment could be conducted with multi-location in both seasons. In this study 5% of seaweed extract was used therefore different formulation of seaweed extract with varying concentration could also be tried in future studies.

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# ATTITUDE OF YOUNG GENERATION ON CINNAMON CULTIVATION AND PROCESSING: A CASE STUDY IN KARANDENIYA DS DIVISION

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## Introduction

Cinnamon in Sri Lanka has a long history and strong reputation of production of intrinsic and inherent high quality all over the world. Cinnamon provides direct impact to the economy while extending indirect impacts to the social, political and technological environments. The importance of the industry has significantly increased after it directly connected with the social life of the community. In recent years the cinnamon industry is declining, even though there is a positive growth in the export market. Some of the probable reasons that affect this situation are, shifting away of new generation from the cinnamon industry, lack of trained labor, High cost of labor, Lack of technical knowledge, and prevailing social stigma against the sector.

From the above mentioned factors shifting away of young generation from the cinnamon industry is a significant issue in our context. Nowadays the contribution of young generation in cinnamon cultivation and processing is relatively low. As a result of this, the cultivators have to bear a high cost of labor which is increasing day by day due to the lack of trained labor. This leads the cultivators to a low profit. If young generation can help their parents in cultivation and processing, the profit can be increased by decreasing labor cost. There are lack of research investigation to see young generation's perception on cinnamon cultivation and processing. Therefore it is timely important to consider the perception of the young generation regarding the cinnamon cultivation and processing to uplift cinnamon industry of Sri Lanka.

The main aim of the study is to identify the perception of the young generation on cinnamon cultivation and processing. In order to achieve the main aim, several specific objectives were developed. Those are to examine the perception of the young generation on cinnamon cultivation and processing and to identify the problems related to low involvement of young generation in cinnamon cultivation and processing

## Materials and Methods

*Decline of the cinnamon industry*

Rupasinghe [3] said that even though the cinnamon industry performs with a slight positive growth in the export market, the cinnamon cultivated land extent

had declined over the past decades. Nowadays it is mostly seen as small scale cultivation units within the industry as a result. The total production and the average yield have been decreased without a considerable change in the extent of cultivation during the last two decades. However, the comparatively lower quality standards of Ceylon Cinnamon were a reason obstructing the demand for Ceylon Cinnamon in the global markets. The chronic dearth of trained labor in the local spice industry and the prevailing social stigma against the sector has been the main obstacles to the development of Ceylon Cinnamon as a major export. During the recent decades the motivation and the consideration towards the cinnamon industry among the cultivators also has diminished.

*Limitations regarding cinnamon cultivation*

The final market price of the Cinnamon Quills is decided by the existing buyers at most of the time. This is mainly because these few buyers had developed high bargaining power over the situation and well organized to match the situation. Since price is the major factor directly affecting the progress the current situation is discouraging them to stay in the industry. Because the low margins they receive will not be enough even for carrying out the next cycle of harvesting. Therefore it hinders the sustainable development of the industry as planned.

Thanthrige [1] has revealed that in the plantation sector one major issue is that most of the trees/ bushes are very old. Some are even more than few centuries years old. So it needs to be replanted with the assistance from the necessary institutes and incorporate the newly developed species of Cinnamon which provide increased yield. Zoysa [2] found main problems faced by the cinnamon growers at present can be given as follows. Lack of basic economic infrastructure, High production cost, Shortage of skilled labor, Low labor productivity, Lack of rural credit. Rupasinghe [3] identified the major problems among cinnamon growers are the number of holdings that has to be covered by the extension approach, availability of skilled, seasonal labor, availability of inputs, limited number of extension personal and funds, assistance package, poor organizational pattern of the growers' lack of market information and the extension coverage.

*Methodology*

After identifying the research problem, Stimulus response model was used as the suitable conceptual model to find out the solution for the problem. Both primary and secondary data were collected in this study. Primary data were collected directly by Personnel interview of respondents using a pre tested questionnaire, direct observations and conducting formal and informal conversations with the group members, different stakeholders. Secondary data were collected from internet, magazines, books, journals, newspapers. Purposively sampling technique was used and sample size was 150 including 50

cinnamon cultivators, 50 processors and 50 young children of those cultivators and processors in Karandeniya divisional secretariat division.

Data analyzed using inferential and appropriate statistical tests. Since data were not normally distributed, Wilcoxon signed rank test was used to analyze the respondents' data. There were two main hypotheses tested in this study.

Hypothesis 1

$H_0$ - There is a significant relationship between the perception of young generation and the fact $X_i$.

$H_1$- There is not a significant relationship between the perception of young generation and the fact $X_i$.

Hypothesis 2

$H_0$– There is a significant relationship between the perception of young generation and the problem $Y_i$.

$H_1$ – There is not a significant relationship between the perceptions of young generation problem $Y_i$.

$X_1$-cultivation easy
$X_2$ - processing easy
$X_3$ - cultivation costly
$X_4$ - processing costly
$X_5$- desire to engage cultivation
$X_6$ - desire to engage processing
$X_7$ - practice the cultivation is easy
$X_8$ - receive sufficient income from cultivation
$X_9$ - processing is good income source
$X_{10}$ - cultivation take long time to harvest
$X_{11}$ - processing take long time to receive money

$Y_1$-technology not sufficient for cultivation
$Y_2$-technology not sufficient for processing
$Y_3$-hard to take knowledge for cultivation
$Y_4$-hard to take knowledge for processing
$Y_5$-not have initial capital for cultivation
$Y_6$-not receive financial support for cultivation
$Y_7$-not receive financial support for processing
$Y_8$- not have skill for processing
$Y_9$- processing not socially acceptable
$Y_{10}$-no knowledge dissemination programs
$Y_{11}$- no training programs for processing
$Y_{12}$-no government assistance for cultivation
$Y_{13}$- no policies for processing
$Y_{14}$- cultivation not attractive
$Y_{15}$- processing not attractive
$Y_{16}$- health hazards from processing

$Y_{17}$- not like to bad odor of processing
$Y_{18}$- processing disturbs physical appearance
$Y_{19}$- not enough extension

These $X_i$ and $Y_i$ are variables of Stimulus response model.

**Results and Discussion**

According to Table 1 since all P-value<0.05, therefore the null hypotheses for each factors are rejected at 5 percent level of significant. This concludes that there are significant relationships between the all factors and the perception of young generation on cinnamon cultivation and processing.

**Table 1.** The results gained from the Wilcoxon sign rank test

| Facts | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Asymp. Sig.(2-tailed)** | .000 | .000 | .000 | .000 | .001 | .000 | .000 | .000 | .000 | .000 | .000 |

According to Table2 since all P-value<0.05, therefore the null hypotheses for each suggested problems are rejected at 5 percent level of significant. This concludes that there are significant relationships between the all problems and the perception of young generation on cinnamon cultivation and processing.

**Table 2.** The results gained from the Wilcoxon sign rank test under identifying existing problems or limitations regarding the involvement of young generation on cinnamon cultivation and processing

| $Y_i$ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Asm. Sig. (2-taile)** | 0.002 | 0.000 | 0.033 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

| $Y_i$ | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|
| **Asymp. Sig.(2-tailed)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

**Conclusions**

According to the results of Wilcoxon signed rank test considered all problems faced by the cinnamon cultivators are significantly depend on the cinnamon cultivation and processing. The problems $Y_1$ and $Y_2$ are about technological errors. Therefore, improvement of new technology and replace the old

equipments with new machines and equipments can be reduce the $Y_1$ and $Y_2$ problems. $Y_3$, $Y_4$, $Y_8$, $Y_{10}$ and $Y_{11}$ can be resolved by improvement of training programs and organizing awareness programs about the cultivation. Credit support from private sector can be avoided the problems $Y_5$, $Y_6$ and $Y_7$. Other suggested problems can be mostly reduced by the government encouragement, extension services and launching new safety systems.

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# MARKER *ABUOP0003*: A MODIFIED MOLECULAR MARKER FOR GEL-BASED GENOTYPING OF SUBMERGENCE TOLERANCE GENE *SUB1A* IN RICE

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## Introduction

With drastic climate changes, unpredictable flashfloods have become a major constraint on rice cultivation especially in rain-fed lowlands in Asia and Africa [1]. Annually it's been recorded that 25% of the global rice lands are inundated by flood water. Most of the rice varieties are unable to survive in fully-submerged conditions for more than three days. This leads to an economic loss of more than one billion US dollars per annum [2]. However, a variety with submergence tolerance can withstand total submergence for a prolong period, even up to two weeks, and still regrow when the water level depletes.

The tolerance to submergence is governed by several quantitative trait loci (QTL). The major QTL *Sub1* mapped to the rice chromosome 9 was identified as the main determinant which confers the submergence tolerance. Within the *Sub1* region three causal genes were identified, namely *Sub1A, Sub1B* and *Sub1C.* The *Sub1A-1* allele of *Sub1A* gene is known to convey tolerance to submergence, while the *Sub1A-2* allele convey intolerance in rice [5]. In order to detect the genetic tolerance conferred by *Sub1A-1*, several molecular tools have been used. Some of these markers were such as dominant markers [4, 5] and some adopted time-consuming assays such as restriction fragment length polymorphism (RFLP) markers and cleaved amplified polymorphic sequence (CAPS) markers [2]. While high throughput marker assays such as Kompetitive allele specific PCR (KASP) markers targeting the mitogen-activated protein kinase (MAPK) site in the *Sub1A* region are available, however, the use of such markers are hindered in resource-limited countries [2]. For such technology-limited countries a gel-based PCR assay which is accurate, cost effective, and co-dominant would be ideal. Here, we report the development of a gel-based molecular marker, through a modification to the published *AEX1* marker[2], targeting the MAPK site in the *Sub1A* gene for the rapid and cost effective detection of *Sub1A1* and *Sub1A2* alleles.

## Materials and Methods

*Plant materials*

Nine rice accessions were selected based on the presence/absence of *Sub1A* alleles as reported in previous studies [2, 4]. All breeder seed stocks were sourced from the Regional Rice Research and Development Institute (RRRDI), Bombuwala, Sri Lanka and Rice Research and Development Institute, Bathalagoda, Sri Lanka. The DNA was extracted from the rice accessions using a modified CTAB method.

*Development of the molecular marker ABUOP003*

Based on Xu et al 2006, the genomic sequences of IR40931 carrying *Sub1A-1* (GenBank accession number: DQ011598), a descendant of the submergence tolerant line FR13A were retrieved from the National Center for Biotechnology Information (NCBI) repository, and the *Sub1A* genomic region of the *Oryza sativa* subsp. *indica* reference accession Cultivar 93-11 carrying *SUB1A-2* was retrieved from the Gramene Database (Gene ID: BGIOSGA038325). The retrieved sequences were aligned using the ClustalW feature in Geneious v.7.1.3. The target region was identified with the annotation of the *AEX1* marker[2] and the MAPK site carrying the T/G single nucleotide polymorphism (SNP) was targeted for marker development. Based on the allele specific AEX1_R primer (herewith referred to as *ABUOP0003_R1*), a second reverse primer (herewith referred to as *ABUOP0003_R2*) was designed targeting the alternative allele call at the MAPK site using the Primer3 feature in Geneious v7.1.3. The two reverse primer pairs (*ABUOP0003_R1*/*R2*), along with a common forward primer (*ABUOP0003_F*) was assayed on a panel of nine submergences tolerant (FR13A and IR119) and intolerant (Nipponbare, Cultivar 93-11, IRBB21, IR42, IR64, Swarna and Samba Mahsuri) rice accessions.

*Validation of the marker ABUOP0003*

The PCR amplification was carried out in a 15 µl final PCR reaction volume that includes 50 ng/µl template DNA, 1× GoTaq® Green master mix (Promega-USA), 0.33 µM concentration of common forward primer *ABUOP0003_F* and reverse primer *ABUOP0003_R1* at a 0.33 µM concentration or the reverse primer *ABUOP0003_R2* at a 0.25 µM concentration in a thermal cycler (CT1000, Bio-Rad, USA). In the no-template control, the template DNA was replaced with nuclease free water (Promega – USA). The amplification was conducted with an initial denaturation at 95 ˚C for 5 min: 30 s denaturation at 95 ˚C, 30 s primer annealing at 62 $^{o}$C and 1 min primer extension at 72 ˚C for 35 cycles followed by a final extension of 72 ˚C for 5 min. The products were resolved on a 3% agarose gel pre-stained with 5% (v/v) ethidium bromide to visualize and capture the profile through the gel documentation system (Model UVCI-1100, Major Science, USA).

**Results and Discussion**

The new modified molecular marker *ABUOP0003* is designed to target a SNP polymorphism at the MAPK site of the *SUB1A* region in rice and it is modified in to a codominant assay based on the previously published sequence-tagged dominant marker *AEX1*[4]. The new marker presented here is a three component marker, with two allele-specific reverse primers and one common forward primer. The primer combinations amplify similar sized products of 231 bp from each of the primer combinations *ABUOP0003_F/R1* and *ABUOP0003_F/R2* (Table 1), amplifying fragments from template carrying T and C at the respective MAPK sites.

**Table1.** Primer sequences of the marker *ABUOP0003*

| Primer Name | Sequence |
|---|---|
| *ABUOP0003_F* | AGGCGGAGCTACGAGTACCA |
| *ABUOP0003_R1* | GCAGAGCGGCTGCGA |
| *ABUOP0003_R2* | GCAGAGCGGCTGCGG |

The otherwise dominant marker assay was made codominant by adopting a modification to the gel electrophoresis protocol, where the products coming from the two allele-specific primer combinations for each template DNA were loaded on to the same well of the 3% agarose 10-minutes apart. After the subsequent loading of the products the accessions carrying the PCR amplicons of *ABUOP0003_F/R1*, which is loaded first will be visualized furthest from the wells in the 3% Agrose gel, with no PCR amplicons of *ABUOP0003_F/R2* if the accession is homozygous for *Sub1A1* (Figure 1A). If the accession is carrying homozygous *Sub1A2*, only a PCR amplicon closest to the wells will be visualized in the 3% Agrose gel and no products will be visualized at the furthest end (Figure 1A). If the accession under consideration is a heterozygote for *Sub1A,* both products will be visible making the assay a codominant one (Figure 1A). In the absence of both *Sub1A*, no amplification will be resulted from either primer pairs (Figure 1A). The reported altered method of loading the PCR products of *ABUOP0003* with a deliberate loading interval has resulted the same sized allele-specific PCR products be visualized as a codominant and informative assay.

When the modified marker *ABUOP0003* was assayed on a panel of rice accessions, the marker *ABUOP0003* identified that the accessions FR13A and IR119 were carrying the *Sub1A1* allele by amplifying a product only for *ABUOP0003_F/R1* (Figure 1B). This finding is in agreement with the report of Moon et al (2019) on FR13A and with the personal communications made by the Regional Rice Research and Development Institute, Bombuwala on IR119 where the accession is commonly used as a reference line carrying *Sub1A1* allele in trials. The rice accessions IR64 and cultivar 93-11 amplified only the products of *ABUOP0003_F/R2,* and thereby was complying with the findings of Moon *et*

*al* (2019) and Xu *et a*l (2006) confirming that these accessions were carrying the *Sub1A2* (Figure 1B). Further, the marker *ABUOP0003* could successfully identify the null *Sub1A* allele of previously reported rice accessions Nipponbare, Swarna, Swarna Mahsuri, IRBB21 and IR42[2,4] as no amplification was reported for both primer combinations indicating the absence of the *Sub1A* gene in them (Figure 1B). No heterozygote accessions for the *Sub1A* gene was identified in the current study. The marker *ABUOP0003* is a low-cost gel-based alternative to interrogate the SNP based polymorphism of the MAPK site of the *Sub1A* gene to distinguish the tolerance allele *Sub1A1* from the intolerance allele *Sub1A2* in rice.

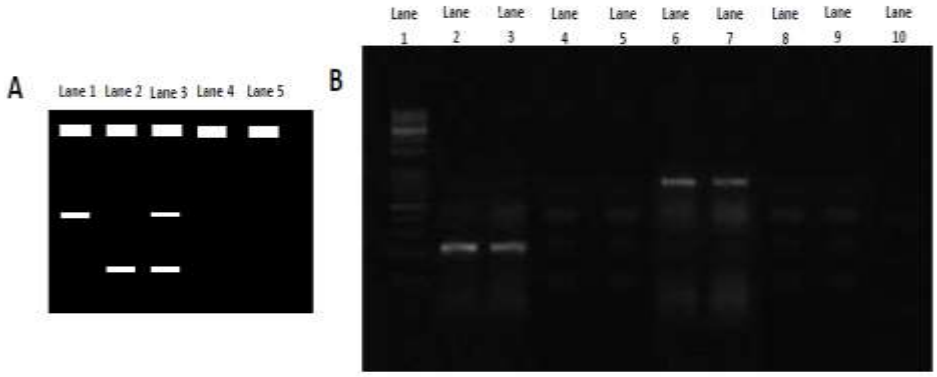


**Figure 1.** A) Illustration of the expected marker profile of *ABUOP0003* Lane 1: *Sub1A1,* Lane 2: *Sub1A2,* Lane 3: Heterozygote, Lane 4: Null allele at *Sub1A* and Lane 5: no template control; B) *ABUOP0003* assay on nine selected rice accessions. Lane1:1kb ladder (Applied Biological Materials-Canada), Lane 2: FR13A, Lane 3: IR119, Lane 4: Nipponbare, Lane 5: Swarna, Lane 6: IR64, Lane7: Cultivar 93-11, Lane 8: Samba Mahsuri, Lane 9: IR42, Lane 10: No template control (NTC)

## Conclusion

The marker *ABUOP0003* can be effectively used as a co-dominant gel-based molecular marker for the identification of *Sub1A1* and *Sub1A2* alleles conveying tolerance and intolerance to submergence in rice, respectively.

## Acknowledgement

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# YIELD DETERMINING PHYSIOLOGICAL AND AGRONOMIC PARAMETERS OF THREE COWPEA VARIETIES GROWN IN ANURADHAPURA, SRI LANKA

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## Introduction

Cowpea (*Vigna unguiculata* L. Walp) also known as southern pea or black-eyed pea is an important pulse crop in the tropics [1]. Total production of cowpea in Sri Lanka was 8,689 metric ton in 2018 with an average yield of 1.16 t/ ha, which is far below its potential yield, 2t/ha [2, 7]. Temperature stress that makes a substantial impact on both growth and yield performance is identified as a major constraint limiting the productivity of cowpea worldwide [1]. Crops show a maximum rate of growth at the optimum temperature, which declines at supra optimal range. The mean ambient temperature often exceeds optimum or falls within supra optimal range and negatively affects on the yield. The optimum temperature for growth and development of cowpea is around 30 °C [3]; however, in dry zone of Sri Lanka, day temperature often exceeds 32 °C. Therefore, we conducted a field experiment to assess the effect of increasing temperature on physiological and agronomic traits of three recommended cowpea varieties in Anuradhapura, Sri Lanka.

## Materials and Methods

*Experimental Site*

A field experiment was conducted at the Field Research Unit, Faculty of Agriculture, Rajarata University of Sri Lanka (81 m amsl, DL1b) during yala season in 2019. The average temperature [(daily min. + max.)/2] of the experimental site was 27.3 °C during the experimental period.

*Data collection*

Three treatments (3 varieties of cowpea; Dawala, Waruni and MI35) were laid according to RCBD design with 4 replicates/treatment. All the cultural practices including irrigation and fertilization were carried out as per the recommendations of Department of Agriculture, Sri Lanka. Seeding rate of 35 kg/ha was used in the study. At 50% flowering stage, leaf gas exchange measurements including light saturated net photosynthetic rate ($A_{sat}$), light

and $CO_2$ saturated net photosynthetic rate ($A_{max}$) and leaf dark respiration ($R_D$) during 0700 to 1300 hrs. of the day were made using a LI-6400 XT portable infrared gas analyzer (Li 6400XT, LiCOR Bioscience, U.S.A.). The temperature of the leaf chamber of the LI-6400 XT was set to 32°C allowing the leaf to achieve a range of temperatures from 30-36°C during measurements.

The total leaf area of the plant at 50% flowering stage, number of plants/$m^2$ at harvesting stage, number of pods/plant at harvesting stage, number of seeds/pod at harvesting stage, 100 seeds weight at harvesting stage were measured as yield determining agronomic parameters. Leaf area was measured using ImageJ software. Moreover, 100 seeds weight at storing moisture level of 12% was measured and grain yield in kg/ha was also calculated.

*Statistical analyses*

Data were analyzed using ANOVA procedure while mean separation of agronomic and physiological parameters was performed using Duncan's multiple range test [4]. Leaf gas exchange values ($A_{sat}$, $A_{max}$ & $R_D$) were fitted with leaf temperature using a quadratic function [5]. Test was also performed to determine whether the grain yield correlate with $A_{sat}$, $A_{max}$ and $R_D$.

**Results and Discussion**

There was significantly higher $A_{sat}$ in varieties Dawala & Waruni compared to MI35 (Table 1). The variety Waruni recorded the highest grain yield as well as the highest $A_{sat}$ in our experiment. Relative to the high $A_{sat}$ of Waruni its $R_D$ was low indicating a high net photosynthetic carbon gain relative to a unit of carbon lost due to leaf respiration (Table 1). With respect to $A_{max}$, MI35 exhibited the highest significant value when compared to other two cowpea varieties tested. However, there was no correlation found between the grain yield and $A_{max}$ or $R_D$ in any variety tested.

**Table 1.** Physiological parameters of different cowpea varieties

| Cowpea variety | Dawala | Waruni | MI35 |
|---|---|---|---|
| $A_{sat}$ (µmol $CO_2$ $m^{-2}$ $s^{-1}$) | 40.39±1.5 [a] | 42.35±0.5[a] | 33.49±0.6[b] |
| $A_{max}$ (µmol $CO_2$ $m^{-2}$ $s^{-1}$ ) | 67.01±1.0 [b] | 70.00±1.8[b] | 82.18±1.8[a] |
| $R_D$ (µmol $CO_2$ $m^{-2}$ $s^{-1}$) | 6.74±0.1[a] | 3.78±0.1[b] | 6.74±0.2[a] |

Means with the same letters in a given row are not significantly different at $p$= 0.05. $A_{sat}$ - light saturated net photosynthetic rate at ambient level of $CO_2$ (400 ppm) and photon flux density at 2200 µmol $m^{-2}$ $s^{-1}$. $A_{max}$- maximum rate of net photosynthesis at 1800 ppm of $CO_2$ and photon flux density at 2200 µmol $m^{-2}$ $s^{-1}$. $R_D$ – Rate of leaf dark respiration

Optimum temperatures for physiological parameters, i.e. $A_{sat,}$ and $A_{max}$, showed no significant differences among the three varieties tested (Figure 1). Therefore, the three cowpea varieties compared in our experiment may not show a difference in physiological responses at higher ambient temperatures. However, optimum leaf temperature for $A_{sat}$ of Waruni was found to be less than that of the other two varieties. The $A_{sat}$ of Dawala and MI35 reduced at leaf temperatures above 34°C. The $A_{sat}$ of MI35 and Dawala seems to continue at higher leaf temperatures than that of Waruni. The $A_{max}$ of Dawala continued at higher leaf temperatures than the other two varieties (Figure 2). All the tested three varieties exhibited increasing rates of $R_D$ with increasing leaf temperature indicating higher carbon loss at higher leaf temperatures (Figure 3).

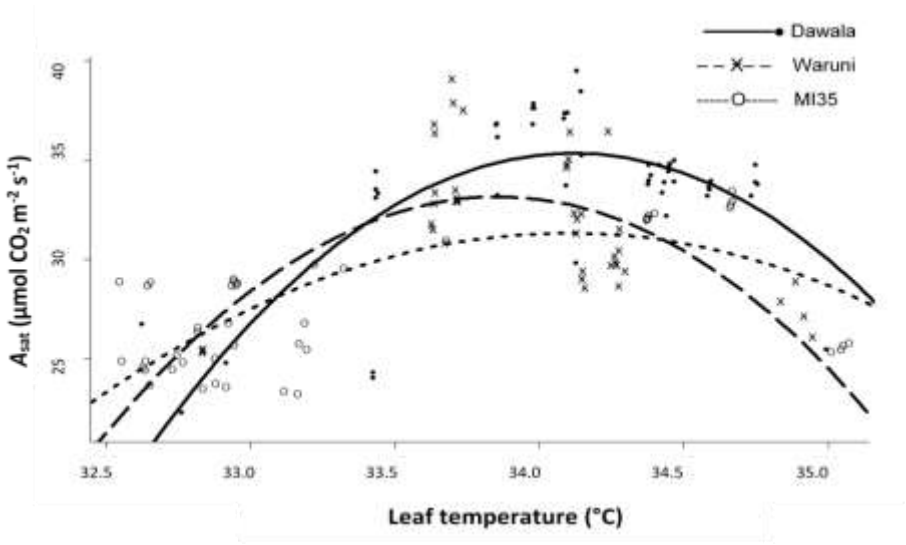


**Figure 1.** Response of $A_{sat}$- maximum rate of photosynthesis at ambient level of $CO_2$ (400ppm) and maximum level of photon flux density (2200 µmol $m^{-2}$ $s^{-1}$) - with leaf temperature of three varieties

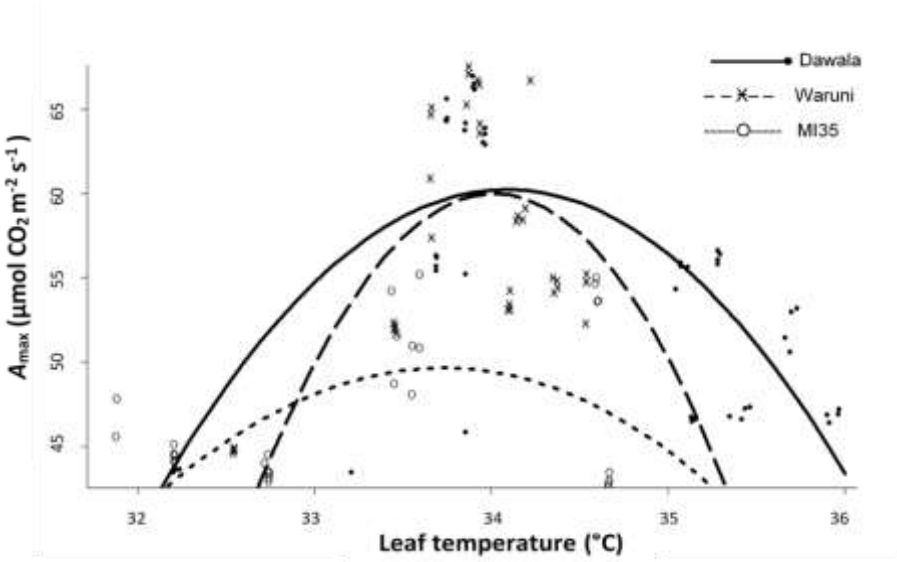


**Figure 2.** Response of $A_{max}$- maximum rate of photosynthesis at 1800 ppm $CO_2$ and maximum level of photon flux density (2200 µmol $m^{-2}$ $s^{-1}$) with leaf temperature of three varieties

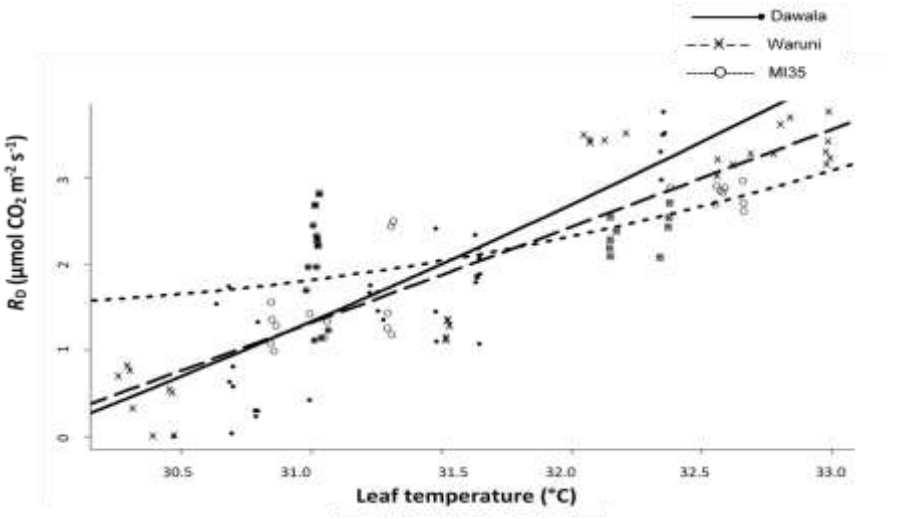


**Figure 3.** Response of $R_D$ – Rate of dark respiration - with leaf temperature of three varieties

With respect to agronomic parameters, Dawala showed significantly higher leaf area at 50% flowering, the number of pods per plant and the number of seeds per pod at harvesting stage than Waruni and MI35. The leaf shape of Dawala must have contributed to exhibit the highest leaf area among three varieties. Generally, seeds of Dawala were larger than other two varieties [7]; however, Dawala seeds showed some size reduction in our experiment. Despite Dawala

having the highest number of seeds per pod, it showed less 100 seeds weight. Furthermore, MI35 had the highest mean value among all agronomic parameters monitored except leaf area. The 100 seeds weight at harvesting stage, 100 seeds weight at storing moisture level (12%) and the grain yield (kg/ha) were significantly different among the three varieties. The highest mean grain yield was showed by the variety Waruni.

Current average yields of cowpea are 1,350 kg $ha^{-1}$, 1,650 kg $ha^{-1}$ and 1,600 kg $ha^{-1}$ for MI35, Waruni and Dawala, respectively as per the Department of Agriculture [6]. However, in this experiment, the average yields recorded were 1256kg $ha^{-1}$ for MI35, 1532 kg $ha^{-1}$ for Waruni and 1178 kg $ha^{-1}$ for Dawala; which were slightly less than the above yields (Table 2). Overall, these results suggest that effects of temperature is prominently related to the physiological performance of these three cowpea varieties however, reflected to a lesser extent in relation to agronomic parameters.

**Table 2.** Variation in agronomic parameters among three cowpea varieties grown in Anuradhapura, Sri Lanka

| **Cowpea Variety** | **Dawala** | **Waruni** | **MI35** |
|---|---|---|---|
| Leaf area ($cm^2$) | $140.3\pm1.8^a$ | $130.9\pm2.5^b$ | $126.0\pm0.4^b$ |
| Number of plants per $m^2$ | $12\pm0.2^b$ | $16\pm0.7^a$ | $16\pm0.4^a$ |
| Number of pods per plant | $14.2\pm0.4^a$ | $8.8\pm0.4^b$ | $9.8\pm0.4^b$ |
| Number of seeds/ pod | $13.3\pm0.2^a$ | $11.2\pm0.2^b$ | $11.0\pm0.1^b$ |
| 100 Seeds weight at harvesting (g) | $9.5\pm0.1^c$ | $10.4\pm0.0^b$ | $12.3\pm0.0^a$ |
| 100 Seeds weight at 12% moisture (g) | $8.2\pm0.1^c$ | $9.0\pm0.0^b$ | $10.2\pm0.0^a$ |
| Grain yield (kg $ha^{-1}$) | $1178\pm94^b$ | $1532\pm72^c$ | $1256\pm137^a$ |
| Current average yield (kg $ha^{-1}$ ) | 1,600 kg $ha^{-1}$ | 1,650 kg $ha^{-1}$ | 1,350kg $ha^{-1}$ |

Leaf area measurements were taken at the 50% flowering stage. Mean values of agronomic parameters of three varieties are shown in the columns. Means with the same letters given in a row are not significantly different at $p = 0.05$.

## Conclusions

There are varietal differences ($p \leq 0.05$) in leaf area at 50% flowering, the number of pods per plant at harvesting stage and the number of seeds per pod at harvesting stage, 100 seeds weight at harvesting stage, 100 seeds

weight at storing moisture level (12%) and the grain yield in kilograms per hectare. The average yields of tested varieties were less than those indicated by the Department of Agriculture, which could be attributed to the soil & climatic conditions prevailed in the dry zone during the experimental period. Even though MI35 and Waruni performed physiologically better than Dawala variety at higher temperatures, further studies on acclimation potential of these varieties under different growth temperatures for the whole growth period of the crop is needed before drawing any solid conclusions from the current study.

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# FOCUS AREA

## Health

# GENDER DIFFERENCES OF VITAMIN D LEVELS AND ITS ASSOCIATION WITH SELECTED OBESITY MARKERS, INSULIN RESISTANCE IN YOUNG ADULTS IN SRI LANKA

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## Introduction

Vitamin D is involved in many metabolic functions of human body. Vitamin D is supplied to the body from diet, dietary supplement and sunlight. It was believed that vitamin D deficiency is less prevalent in countries with rich sunlight, however recent researchers have demonstrated the opposite [1].

Sri Lanka is a country with plenty of sunlight almost thought the year. However, it has been found that there is a high prevalence of vitamin D insufficiency in countries which lies near the equator as well. On the other hand young populations of Sri Lanka are at risk of getting metabolic diseases including Type 2 diabetes in early stages of their adulthood due to increased prevalence of obesity and unfavorable life styles.

Vitamin D deficiency can occur due to many reasons. Obesity is considered as one reason. Previous studies have demonstrated inverse relationship with markers of body fat stores and vitamin D levels. Since vitamin D is a fat-soluble vitamin, adiposity reduce the bioavailability of vitamin D [2]. Since there is a difference in body composition between males and females there could be a gender difference of vitamin D levels as well.

Vitamin D has become a trending research interest, because previous studies have demonstrated that vitamin D insufficiency play a role in the development of cancers, hypertension, autoimmune diseases, diabetes, cardiovascular diseases and many other disease conditions [2].

Based on above facts the objectives of the study were formulated. Objectives of current study were to determine the gender differences of vitamin D status, to determine the association of vitamin D with selected markers of obesity including body fat levels and Body Mass Index (BMI) and to determine the association of vitamin D levels with markers of glucose homeostasis of the body among a young adult population in Sri Lanka.

## Materials and Methods

Hundred young adults from age 18 – 35 years were selected for the current study (female subjects-63) who currently reside in Colombo district for educational and occupational purposes.

*Sample collection*

A written informed consent was collected from the each volunteer. Ten hour fasting blood samples were collected for the determination of biochemical parameters including fasting blood glucose levels (FBS), fasting insulin levels and serum vitamin D levels. Interviewer administered questionnaire was given for the collection of socio-demographic data.

*Biochemical analysis*

Fasting blood glucose levels were determined using the glucose oxidatse method. Fasting insulin levels were determined using Enzyme Linked Immunosorbant Assay. The 25-Hydroxyvitamin D levels were determined using Minividas-immunoanalyser. 25(OH) D levels > 20 ng/ml were considered as sufficient levels and < 20 ng/ml were considered as inadequate levels following Institute of Medicine (IOM) guidelines [3]. Deficient (<10 ng/ml) and inadequate (10 -20 ng/ml) categories were combined for the feasibility of data analysis. Homeostatic Model Assessment Equation (HOMA) was used to determine the Insulin Resistance (IR).

*Assessment of body composition*

Anthropometric measurements were taken following World Health Organization guidelines to calculate the body mass index (BMI). Omron bio-impedance analyzer (BIA) was used to determine the body fat percentage (BF %), Subcutaneous fat percentage (SF %), Skeletal Muscle percentage (SM %) and visceral fat levels.

*Data analysis*

Data analysis was performed using SPSS version 16.0 statistical software package. Parametric and non-parametric statistical methods were applied for the statistical analysis based on the distribution of the variables. Mean values were reported as the data of central tendency although some measured parameters were not normally distributed.

## Results and Discussion

Mean age of the study population was 28 years old. Majority of the study population were undergraduates (75%) and rest of the population were employed (25%).

Based on the classification criteria, majority (66%) of the population belonged to the inadequate category (66% of females and 67% of males) of serum Vitamin D levels.

A previous study which was conducted on a Israel population demonstrated that 78% of the population with vitamin D insufficiency [4]. Another study which was conducted on an American young adult population (age 17-35 years) demonstrated that 36% of the population were vitamin D insufficient towards the end of the winter demonstrating that there is a seasonal variation as well [5].

A study which was conducted in a population of pregnant women in Sri Lanka reported that Prevalence of vitamin D deficient/insufficient group was 63%, which was almost same for the current population as well. This study also used IOM guidelines for the classification of vitamin D status. On the other hand the mean vitamin D value of the pregnant women population was 18.7 (±7.2)(ng/mL) which was coincidently similar for the current study population as well [3].

Although mean FBS and fasting insulin levels were with in normal ranges, mean HOMA-IR values were above the cutoff values for insulin resistance. This might be due to non-normal distribution of the variables.

**Table1.** Mean values of body composition and biochemical parameters of the population

| Parameter | Whole population (100) | Females (63) | Males (37) | P value (Females and Males) |
|---|---|---|---|---|
| BMI ($kgm^{-3}$) | 23.0 (±4.3) | 22.1 (±3.7) | 24.4 (±4.7) | <0.05 |
| BF% | 31.8 (±29.6) | 36.3 (±18.8) | 24.1 (±5.4) | <0.05 |
| SC % | 22.7 (±6.1) | 26.1 (±4.1) | 16.9 (±4.4) | <0.05 |
| SM % | 32.9 (±30.1) | 28.7 (±17.6) | 40.1 (±17.2) | <0.05 |
| Visceral fat level | 5.6 (±4.4) | 4.1 (±2.8) | 8.4 (±5.1) | <0.05 |
| FBS (mg/dl) | 80.5 (±11.0) | 82.3 (±8.9) | 77.4 (±13.3) | >0.05 |
| Fasting insulin level (µIU/ ml) | 10.2 (±6.2) | 8.8 (±4.1) | 12.6 (±8.2) | <0.05 |
| IR | 2.5 (±1.8) | 3.3 (±2.8) | 2.5 (±1.9) | >0.05 |
| Vitamin D (ng/ml) | 18.7 (±5.9) | 18.6 (±5.4) | 18.8 (±6.8) | >0.05 |

BF%= Body Fat Percentage; SC%= Sub-cutaneous Fat Percentage; SM%= Skeletal Muscle Percentage; FBS= Fasting Blood Glucose; IR= Insulin Resistance; IQR= Inter-Quartile Range

There was no statistically significant difference in serum vitamin D levels among female and male groups ($P<0.05$) and the mean values of both groups were within the inadequate levels of vitamin D. There were previous studies which

have demonstrated contradictory findings related to gender differences of vitamin D levels [4].

BMI, BF%, SC%, SM%, visceral fat level, and fasting insulin level showed statistically significant differences between male and female groups.

Among the whole population 4 subjects had impaired fasting blood glucose levels, 4 subjects had high fasting insulin levels and 28 subjects had insulin resistance based on the cutoff values.

**Table2.** Correlations of body composition parameters and biochemical parameters with Fasting blood glucose (FBS), Fasting Insulin levels, Insulin Resistance (IR) and vitamin D levels in the female population

| | **BMI** | **BF %** | **SF %** | **SM %** | **Visceral fat** | **FBS** | **Fasting Insulin** | **IR** |
|---|---|---|---|---|---|---|---|---|
| | CC (Sig.) | CC (Sig.) | CC (Sig.) | CC (Sig.) | CC (Sig.) | CC (Sig.) | CC (Sig.) | CC (Sig.) |
| **FBS** | 0.090 | 0.170 | 0.100 | -0.120 | 0.060 | | | |
| | (0.47) | (0.18) | (0.44) | (0.34) | (0.62) | | | |
| **Fasting Insulin** | **0.548** | **0.365** | **0.378** | -0.140 | **0.450** | -0.110 | | |
| | **(0.00)** | **(0.00)** | **(0.00)** | (0.27) | **(0.00)** | (0.37) | | |
| **IR** | **0.552** | **0.412** | **0.434** | **-0.273** | **0.489** | 0.160 | **0.635** | |
| | **(0.00)** | **(0.00)** | **(0.00)** | **(0.03)** | **(0.00)** | (0.22) | **(0.00)** | |
| **Vitamin D** | -0.210 | **-0.257** | **-0.263** | 0.140 | -0.180 | -0.170 | -0.090 | -0.120 |
| | (0.10) | **(0.04)** | **(0.04)** | (0.28) | (0.15) | (0.18) | (0.51) | (0.34) |

CC = Correlation Coefficient; Sig. = Significance (2-tailed)

**Table3.** Correlations of body composition parameters and biochemical parameters with Fasting blood glucose (FBS), Fasting Insulin levels, Insulin Resistance (IR) and vitamin D levels in the male population

| | **BMI** | **BF %** | **SC %** | **SM %** | **Visceral fat** | **FBS** | **Fasting Insulin** | **IR** |
|---|---|---|---|---|---|---|---|---|
| | CC (Sig.) | CC (Sig.) | CC (Sig.) | CC (Sig.) | CC (Sig.) | CC (Sig.) | CC (Sig.) | CC (Sig.) |
| **FBS** | 0.303 | 0.228 | 0.208 | -0.248 | **0.339** | | | |
| | (0.07) | (0.18) | (0.22) | (0.14) | **(0.04)** | | | |
| **Fasting insulin** | **0.381** | **0.430** | **0.408** | **-0.392** | **0.406** | **0.396** | | |
| | **(0.02)** | **(0.01)** | **(0.01)** | **(0.02)** | **(0.01)** | **(0.02)** | | |
| **IR** | **0.411** | **0.403** | **0.375** | **-0.371** | **0.453** | **0.638** | **0.940** | |
| | **(0.01)** | **(0.01)** | **(0.02)** | **(0.02)** | **(0.01)** | **(0.00)** | **(0.00)** | |
| **Vitamin D** | **-0.333** | **-0.357** | **-0.322** | 0.251 | **-0.388** | -0.111 | -0.199 | -0.202 |
| | **(0.04)** | **(0.03)** | **(0.05)** | (0.13) | **(0.02)** | (0.51) | (0.24) | (0.23) |

C = Correlation Coefficient; Sig. = Significance (2-tailed)

Vitamin D levels of female population was able to demonstrate weak negative significant ($p<0.05$) correlations with BF% and SC%. On the other hand vitamin D levels of male population was able to demonstrate weak to moderate negative significant correlations with BMI, BF%, SC and visceral fat levels ($p<0.05$). There are previous studies which have demonstrated the similar pattern [2].

However fasting blood glucose, fasting insulin levels and insulin resistance of male and female groups did not show any significant correlations with vitamin D levels. This incompatibly might be due to les number of participants in the current study population.

**Conclusions and Recommendations**

Based on the findings of the current study it can be concluded that the current study population is suffering from vitamin D inadequacy irrespective of the gender. And, current population demonstrated that vitamin D is associated with the selected markers of the obesity. However, the current population did not show any significant correlations with vitamin D and insulin resistance.

It is recommended to repeat the study with a population which represents the whole country in order to generalize the research finding. And, it is important to educate the young adult population of Sri Lanka about the importance of vitamin D and its health implications in order to minimize the burden of them suffering from metabolic diseases in the future.

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# NEPHROPROTECTIVE EFFECT OF SELECTED EXTRACTS OF *Barleria prionitis* Linn. AGAINST ADRIAMYCIN INDUCED NEPHROTOXICITY IN WISTAR RATS

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## Introduction

*Barleria prionitis* Linn. (Family; Acanthaceae), commonly known as Katukarandu, is being used in the treatment of kidney diseases in the indigenous system of medicine in Sri Lanka [1]. Various parts of the plant including leaf, stem, root, bark, and flower are being used for the management of kidney-related diseases by Ayurvedic practitioners [2]. However, despite the traditional use, the scientific scrutinization of the plant in the treatment of kidney diseases has not been reported to date. Hence, the present study was undertaken with the intent of evaluating the protective effects of the hexane, ethyl acetate, butanol and aqueous extracts of *B. prionitis* whole plant against adriamycin-induced nephrotoxicity in Wistar rats.

## Materials and Methods

*Ethical clearance*

Ethical clearance was obtained from the Ethical Review Committee, Faculty of Medicine, University of Ruhuna (14.12.2015:3.1).

*Authentication*

The botanical identity of *B. prionitis* was confirmed by comparing it with the authentic samples at National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka.

*Preparation of plant extracts*

The whole plant of *B. prionitis* were dried at 40°C to a constant weight and coarsely ground. Powdered plant materials were sequentially extracted with hexane, ethyl acetate, butanol and distilled water by soxhlet extraction method. The extraction procedure was continued until the extract gave no colouration. Solvents in hexane, ethyl acetate and butanol extracts were concentrated by evaporation under reduced pressure in a rotary evaporator until two-thirds of the initial volume was removed. The resulting semisolid masses were vacuum dried using a vacuum oven. The concentrated aqueous extracts were finally

freeze-dried at -20°C to obtain the lyophilised powder of the selected medicinal plant.

*Induction of nephropathy to rats*

Overnight fasted (8h) healthy male Wistar rats (150±25g) were intraperitoneally injected with adriamycin (5 mg/kg b. wt.) for the induction of nephrotoxicity.

*Experimental design*

Wistar rats were randomly divided into seven groups (n=6/group). Group 1 rats served as untreated healthy control and Group 2 rats served as adriamycin-induced nephrotoxic control received distilled water daily. Group 3 to 6 were adriamycin-induced nephrotoxic rats administered daily with the plant extracts (hexane: 25 mg/kg, ethyl acetate: 80 mg/kg, butanol: 70 mg/kg, and the aqueous: 120 mg/kg; human equivalent therapeutic dose). Group 7 served as the positive control and the nephrotoxic rats were administered with the standard drug; fosinopril (0.09 mg/mL/kg b. wt.). The treatments were initiated 24 hours following the induction of nephrotoxicity. The plant extracts and the standard drug were administered daily as a single dose for 28 days.

Twenty four hour urine samples were collected in diuresis cages at the end of the intervention. The animals were sacrificed at the end of 28 days and a blood sample (3.0 mL) was collected by the cardiac puncture for the estimation of biochemical parameters. The kidney tissues were excised for the assessment of histopathology.

*Assessment of serum kidney function markers*

The serum concentrations of total protein, albumin, blood urea nitrogen, creatinine and urine total protein concentration were estimated using spectrophotometric assay kits.

*Semi-quantitative assessment of histopathology in kidney tissue*

Hematoxylin and eosin-stained kidney sections were examined for the features of tubular cell injury; vacuolar change, loss of brush border and pyknosis in renal tubular epithelium. Intertubular haemorrhage, glomerular congestion and formation of hyaline casts were the additional features observed.

*Statistical analysis*

Data were statistically analysed using SPSS software 22.0 for Windows. One-way ANOVA followed by the least significant difference (LSD) test was used for multiple comparisons of biochemical data.

**Results and Discussion**

A significant increase in serum concentration of creatinine, blood urea nitrogen, urine total protein and a significant decrease in serum total protein and albumin

were observed in the adriamycin-induced nephrotoxic control group compared to the normal control group ($p<0.05$). Those results substantiate that, adriamycin exerts significant nephrotoxicity in rats ($p<0.05$). As shown in Figure 1, the treatment with the hexane, ethyl acetate, butanol and aqueous extracts of *B. prionitis* decreased the elevation of concentrations of serum creatinine (19%, 26%, 24% and 31%) and blood urea nitrogen (21%, 29%, 24% and 35%) in adriamycin-induced nephrotoxic rats respectively ($p<0.05$). A significant reduction in urine total protein concentration was observed only in the experimental rats treated with the hexane (59%), ethyl acetate (60%) and butanol (63%) extracts ($p<0.05$). A reduction of 22% was observed in experimental rats treated with the aqueous extract of *B. prionitis* however, the change was not statistically significant ($p>0.05$). Moreover, administration of the selected plant extracts reduced the decrease in serum concentration of albumin (47%, 27%, 14% and 31%) and total protein (47%, 38%, 34% and 45%) respectively ($p<0.05$). The highest activity was observed in the butanol extract of *B.prionitis*. Similar changes were observed in the fosinopril treated rats compared to adriamycin-induced nephrotoxic group.

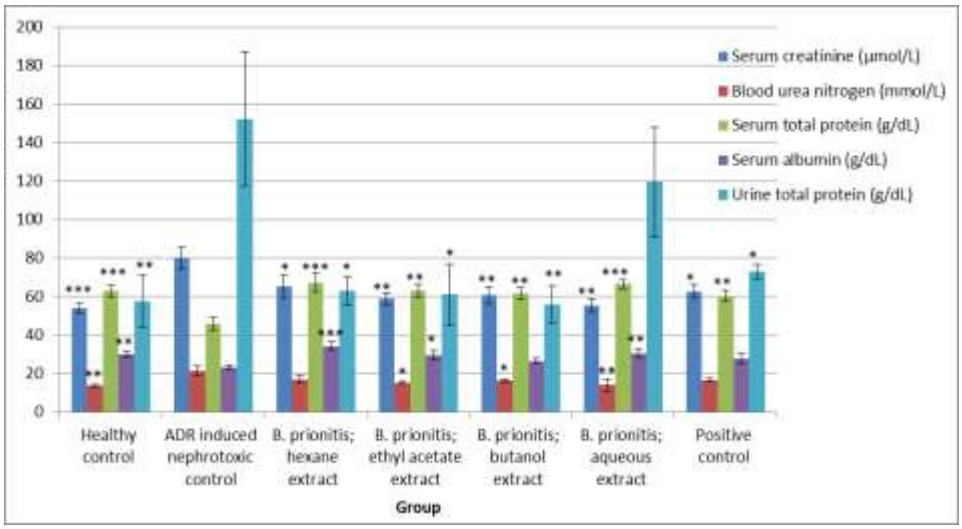


**Figure 1.** The effect of hexane, ethyl acetate, butanol and aqueous extracts of *B.prionitis* whole plant on selected kidney function parameters in adriamycin induced nephrotoxic rats. The results are in mean ± SEM. $*p < 0.05$, $** p < 0.01$, and $***p < 0.001$ compared with normal control group

In the present study, induction of nephrotoxicity with a single intraperitoneal dose of adriamycin produced features of acute tubular cell injury in experimental rats indicating nephrotoxicity. The degenerative tubules showed loss of tubular brush border, nuclear pyknosis and vacuolar change in tubular epithelial cells. Intertubular haemorrhage, glomerular congestion and formation of hyaline casts were the associate features observed. The histopathological

findings corroborated the results of biochemical parameters. The concurrent administration of the plant extracts and the standard drug resulted in attenuation of adriamycin-induced renal tubular and glomerular alterations in experimental rats.

**Conclusions and Recommendations**

The overall results of the present study revealed that hexane, ethyl acetate, butanol and aqueous extracts of *B. prionitis* whole plant possess significant nephroprotective activity against adriamycin-induced nephrotoxicity in Wistar rats. The plant could be a potent source for the development of pharmaceutical agents/formulation in the management of kidney diseases in the future.

**Acknowledgement**

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# ASSOCIATION BETWEEN FLUID AND DIETARY NON-ADHERENCE WITH MORBIDITY AND MORTALITY IN HAEMODIALYSIS PATIENTS ATTENDING TO NEPHROLOGY UNIT, NATIONAL HOSPITAL, KANDY

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## Introduction

Haemodialysis has been developed as a viable, safe and efficient method which is a form of long-term renal replacement therapy used to remove waste products and fluids from the blood of End Stage Renal Disease (ESRD) patients. It helps to control blood pressure and to maintain the proper electrolyte balance in the body. The estimated global population of ESRD patients undergoing haemodialysis treatment is over 1.1 million with increasing at a rate of 7% per year (3).Although Sri Lankan annual health budget spend high cost to treat the ESRD patients, their mortality rate is alarmingly high due to scarcity of resources and complications.

We aimed to determine the association of fluid and diet non-adherence with morbidity and mortality in haemodialysis patients attending to the Nephrology Unit, Teaching Hospital, Kandy. As the previous literature was not available on similar studies, conducting this type of research will be an eye-opener. Findings of this research will be very effective not only for the optimization of Renal Replacement Therapy (RRT), but also in policy making regarding and dialysis expansion.

## Materials and Methods

*Study design and setting*

A prospective observational study was conducted among 312 HD patients admitted to Nephrology Unit, Teaching Hospital, Kandy since October 2018 to September 2019.

*Data collection*

An interviewer administered data collection form was used to collect the data from the patients. Informed written consent was obtained from the haemodialysis patients participating in the research. Follow-up study was carried out using the same patients after six month to assess the morbidity and mortality. Demographic data was obtained, including name, district, age, sex,

ethnicity, educational level, civil status, monthly income, etiology of ESRD. Assessment of water intake was done using 24 hour recall method.

*Ethical clearance*

Ethical clearance was obtained from Ethics Review Committee of Faculty of Allied Health Sciences, University of Peradeniya and Teaching Hospital Kandy.

*Data analysis*

The data was analyzed using the statistical software SPSS (Statistical Package for Social Sciences). Data analysis was done performed by simple descriptive methods using absolute numbers and percentages. Mainly we used chi-square test to assess the relationship between the categorical variables such as, gender, age, educational level, water intake, Intra Dialytic Weight Gain (IDWG), salt intake, protein intake and mortality.

**Results and Discussion**

A total of 312 HD patients recruited to this study. The mean age of participants was 50.6 (SD=13.0581) years with the youngest participant being 17 years of age and the oldest being 83 years of age. Among those 312 patients, male:female ratio is 9:4. Diabetic nephropathy (38.1%) and chronic hypertension (27.6%) are the highest level of etiological factors of CKD in this study.

In the study group, non-adherence to therapeutic fluid, salt and protein intake were 73%, 46% and 58% respectively. According to the previous literatures in United States and Turkey reported that the fluid non-adherence were 54% and 68% (1;4). Statistically significant relationships were found between water intake and gender (P=0.005), educational level (P=0.042), and protein intake (P=0.000). Males had high fluid intake (Figure 1) and low protein intake compared to females. Moreover, high fluid intake and low protein intake reported among participants who studied below O/L and up-to O/L education than other educational categories. Salt intake of this study group was in a satisfactory level compared to other two factors. More than 50% of patients had high level of IDWG than recommended level which is 0.5 – 1.0 Kg/day. There is a relationship between IDWG and age (P=0.030). High IDWG was more prominent in 51-60 age category.

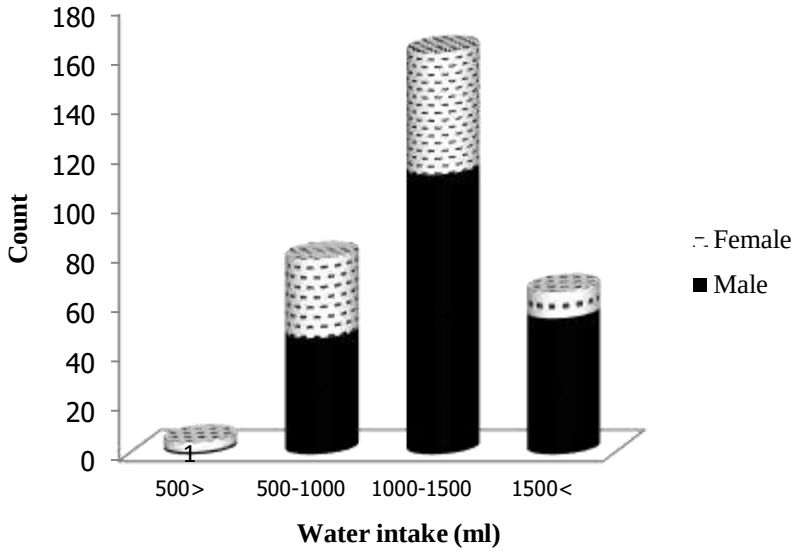


**Figure 1**. Distribution water intake with gender

About 73% of this group has admitted to the hospital during period of 6 months due to various medical conditions. Low protein intake was associated with increase hospital admissions and high fluid intake was the key factor to increase the morbidity symptoms. Loss of appetite and lack of sleep were presented among higher number of patients compared to other morbidity symptoms such as, hypertension, edema, dyspnea and muscle cramps.

In this research study, 76 deaths had reported during the 6 month follow-up period. Hence, mortality rate was 24.35%. Fluid intake >1000ml per day, 3kg <IDWG, Low protein intake and low protein intake can be suggested as crucial factors for increased mortality rate.

**Table 1**. Associated factors for increased mortality rate among HD patients

| Crucial factor | Presence of the crucial factor as a % among 76 deaths |
|---|---|
| 1000ml< water intake per day | 69.7% |
| 3kg< IDWG | 55.3% |
| Low protein intake | 61.8% |
| <250ml of urine output | 68.4% |

A study in Colombia introduced predictable factors for increased mortality in HD patients (2). Age and gender included as traditional factors and anemia and hypoalbunemia suggested as non-traditional factors.

**Table 2.** Compatibility of Colombian study results with current study

| **Factor** | **(Coronado *et al.*, 2016)** | **In this study group** |
|---|---|---|
| Age | Mean age – 66 years | Mean age - 55.51 years |
| Sex | Male- 52.9% | Male -72.4% |
| Anaemia | 30.6% | 77.6% (Low Hb) |
| Hypoalbunaemia | 42.3% | 61.8% |

**Conclusions and Recommendations**

Results of this study proposed that the fluid and dietary non-adherence is attributing to poor outcome of these patients. Lack of knowledge is a major contributing factor among the underlying causes and urgent intervention is warranted. Patient education, identification of at-risk for non-adherence and assisting patients to manage difficulties with life-style changes related to HD are important elements in promoting non-adherence. Special attention to modify these factors will improve the survival of HD patients. Factors such as quality of life (QoL) or the presence of depression could also be considered in future studies.

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# ASSOCIATION BETWEEN SELECTED PARAMETERS INVOLVED IN GLUCOSE HOMEOSTASIS AND SEVERITY OF ANTICHOLINESTERASE INSECTICIDE POISONING

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## Introduction

Organophosphates (OP) and carbamate pesticides are widely used in agriculture as the commonest means of pest control methods especially in developing countries. Poisoning due to pesticides accounts for approximately 3 million cases of hospitalization globally, including 200,000 deaths from OP intoxication per year [1]. Poisoning can be either accidental or intentional. OP act by inhibiting the enzyme acetyl cholinesterase. Symptoms are usually due to muscarinic, nicotinic and central nervous system receptor overstimulation. In addition to those cholinergic manifestations, biochemical changes like glucose dysregulation have also been observed in human and animal studies [2, 3].

Studies have reported altered glucose homeostasis and insulin resistance in acute and chronic exposures to OP, many previous experiments have observed pronounced increase in blood glucose level in parallel with high serum insulin levels as a result of malathion intoxication. The mechanisms involved in the pathophysiology of insulin resistance following OP and carbamate exposure remains under investigations. Some suggested mechanisms include, formation of advanced glycation end products, accumulation of lipid metabolites, activation of inflammatory pathways and oxidative stress.

Many studies have reported that OP impair glucose homeostasis and cause insulin resistance which may lead to the development of type 2 diabetes. Diabetes mellitus has also become an increasing health burden in South Asia with an estimated 82 million affected in 2017 and a predicted population of 151 million in 2045 [2]. Some have suggested exposure to pesticide as one of the contributory factors in the development of diabetes mellitus in agrarian areas. Majority of the supporting evidence are from animal experiments.

Hence, present study was focused to assess the association between selected parameters of glucose homeostasis and the severity of poisoning following intentional ingestion of OP and carbamate insecticide

**Materials and Methods**

This prospective study was carried out with 100 acute OP and carbamate poisoned patients with age ranging from 18-60 years, presenting within 24 hours of poisoning, admitted to Teaching Hospital Anuradhapura. Proxy consent obtained initially and before discharge informed written consent was taken from the patient. Ethical approval was obtained from ethics review committee of faculty of Medical Sciences, University of Sri Jayewardenepura.

The inclusion criteria of the study was patients diagnosed with OP or carbamate poisoning by the clinician according to standard management protocol (Management of poisoning, Prof. Ravindra Fernando) and patients who are admitted within 24 hours of ingestion. The exclusion criteria were, history of poisoning with other toxic substances other than OP and carbamate, pregnant women, subjects with chronic alcoholism, co-ingestion of alcohol and evidence of chronic diseases (diabetes, liver, renal, pancreatic, malignancies etc.).

Data were collected using interviewer administered questionnaire from guardian and patient. First blood sample (5 mL) was collected on admission for analysis of random blood sugar level (RBS) and glycosylated haemoglobin (HbA1c) level.

Severity of poisoning was measured on admission based on the method given as Peradeniya Organophosphorus Poisoning scale (POP). According to POP scale, 0-3 score were considered as mild poisoning, 4-7 as moderate poisoning and 8-11 as severe poisoning.

Second blood sample (5 mL) was collected at the discharge, following 8 – 10 hours of fasting for the analysis of fasting blood glucose (FBS) and fasting serum insulin. Insulin resistance (IR) was calculated using homeostasis model assessment of insulin resistance equation (HOMA-IR). HOMA-IR = {FSI (µU/mL) × FBG (mmol/mL)}/22.5

HOMA -IR above 2.5 were considered as insulin resistance. Data were analyzed using SPSS version 21.

**Results and Discussion**

Subjects (100) with normal HbA1c on admission and no evidence of diabetes were recruited and the majority was males (3). The mean age (SD) of the total population was 33±12 years. Among the study population 45 subjects were identified as organophosphate poisoning while 55 were identified as carbamate poisoning**.**

At the time of discharge mean values (±SD) serum insulin, FBS, and IR were 18 ±16 µIU/ mL, 114 ±36 mg/dL, and 5.7+±6.5 respectively. Mean values of all the biomarkers assessed were significantly elevated in moderate poisoned group compared to mild poisoned group. 11 subjects were included in the severe poisoned group and only 3 survived after treatments, thus the values are not included in the table (Table 01).

**Table 1.** Mean values of assessed biomarkers according to the POP scale

| Parameter | Severity of poisoning based on POP scale | | |
|---|---|---|---|
| | Mild (64) | Moderate (33) | Significance (P value) |
| RBS (mg/ dL) * | 120 ± 39 | 170 ± 58 | 0.000 |
| S. Insulin (µIU/ mL) * | 13 ± 10 | 27 ± 23 | 0.001 |
| FBS (mg/ dL) * | 102 ± 18 | 127 ± 23 | 0.000 |
| HOMA IR * | 3 ± 2 | 9 ± 9 | 0.002 |

Mean values were significantly different at 0.01 level (p<0.01)

Studies have observed an increase in blood glucose levels in parallel with a high insulin secretion into blood through malathion intoxication supporting the findings of current study [4][5][6].

Several studies have also found that insulin resistance is induced by OP insecticides supporting the findings of the current study [7] [8].
The severity of poisoning significantly correlated with the following biochemical parameters; on admission RBS level, and fasting serum insulin level, FBS, HOMA IR measured at the time of discharge. (Table 02).

**Table2.** Correlations between assessed parameters and severity of poisoning according to pop scale

| Parameter | Pearson's correlation coefficient - r | P value |
|---|---|---|
| RBS (mg/ dL) | 0.470 | 0.000 |
| S. Insulin (µIU/ mL) | 0.301 | 0.001 |
| FBS (mg/ dL) | 0.516 | 0.000 |
| HOMA IR | 0.393 | 0.000 |

Correlations were significant at the 0.01 level (p<0.01)

The elevation of FBS, fasting serum insulin and insulin resistance in these patients could be probably associated with activation of inflammatory pathway and oxidative stress.

**Conclusions and recommendations**

Severity of OP and carbamate poisoning was associated with hyperglycaeima, increased insulin and insulin resistance. Further studies will be needed to evaluate the underling mechanism of increased IR and it is important to commence follow-up studies to assess the risk of developing Type 2 diabetes and persistent elevation of HOMA-IR.

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# INCIDENCE OF OVERWEIGHT AND OBESITY VS BODY FAT PERCENTAGES AMONG UNDERGRADUATE STUDENTS OF RAJARATA UNIVERSITY OF SRI LANKA

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## Introduction

Prevalence of overweight, obesity and central obesity among Sri Lankan adults were 25.2%, 9.2% and 26.2% respectively in 2010. Body mass index (BMI) is the most common indicator in establishing the cut-off points of obesity though it is not accurate to use BMI as the sole indicator to predict the risk for metabolic disorders. Different fat depots in different body compartments have different metabolic activity rates. Therefore, fat depots in different compartments contribute to dyslipidaemia and insulin resistance in different ways leading to obesity related complications such as cardiovascular diseases (CVD) and other metabolic disorders. Fat depots differ with ethnicity, age, physical activity levels and diet.

University students are mainly involved in busy schedules of studies, which may reduce the physical activity level and increase consumption of fast food. These factors may increase visceral fat level and metabolic disease risk. Therefore, the objectives of the present study were to assess BMI, total body fat percentage (TBF), visceral fat level and the correlations between BMI and body fat in the students in Rajarata University of Sri Lanka.

## Materials and Methods

This was a cross sectional study of 130 university students of the Rajarata University of Sri Lanka who were selected from the lists of students of the student service centers by simple random sampling (random number table). Following World Health Organization (WHO) guidelines, waist circumference (WC) and hip circumference (HC) were measured using a non-stretchable tape and height was measured using a portable stadiometer (seca213, France). Weight was measured along with body fat measurements by bio-impedance analyzer (BIA) system to the nearest 0.1 kg. BMI was auto calculated in the BIA system, dividing individuals' body weight by height$^2$ in kg/m$^2$ (measured height, age and sex were entered to the BIA initially). Body fat percentage was measured using an 8-electrode BIA system (Karada Scan HBF-375; Omron Corporation, Japan) following instructions in the manual (1). Other obesity

indices; waist to hip ratio (WHR), waist to height ratio (WHtR) and body adiposity index (BAI) were calculated using following formulae;

$$\text{WHR} = \frac{\text{Waist circumference (cm)}}{\text{Hip circumference (cm)}}$$

$$\text{WHtR} = \frac{\text{Waist circumference (cm)}}{\text{Height (cm)}}$$

$$\text{BAI} = \frac{\text{Hip circumference (cm)}}{\text{Height}^{1.5}\text{(m)}} - 18$$

South-Asian cutoff values recommended by WHO were used for anthropometric indices. Values obtained for BAI were classified into low, normal, high and very high levels according to criteria established for Asians by Gallagher et al(2). Cutoff levels in the BIA manual were used for body fat and visceral fat measures (1). Data were analyzed using a beta version of SPSS-23. Ethical approval for the study has been obtained from Ethics Review Committee, Rajarata University of Sri Lanka (ERC/2016/10).

**Results and Discussion**

From the 130 students, 67.7% were females (n=88). Mean age of the sample was 23 ± 1 years. The mean BMI of the present study sample was, 21 ± 3 kg/m$^2$ (same mean was in both females and males). Based on BMI, 14% (11% in females and 19% in males) were overweight and 9% (8% in females and 12% in males) were obese.

The mean waist circumference (WC) was 77 ± 9 cm (74 ± 8 cm in females and 82 ± 9 cm in males). Based on WC, abdominal obesity incidence was 24% (similar incidence was observed in both sex). Means of waist-to-hip ratio and waist-to-height ratio were 0.8 ± 0.1 and 0.48 ± 0.05 (0.47 ± 0.05 in female students and 0.48 ± 0.05 in males) respectively. Central obesity incidence based on waist-to-hip-ratio was 15.4% (17% in females and 12% in males) and waist-to-height was 28% (25% in females and 50% in males). Waist-to-height ratio was considered as a better indicator for cardiometabolic risk assessment (3). Based on waist-to-height ratio 28% of the student population was at high risk of early CVD and other metabolic disorders.

Mean total body fat percentage was 26**%** ± 7 (29 ± 5**%** in females and 19 ± 6% in males). Out of the individuals who were in the normal BMI category 44% had high TBF% while 4% had very high TBF%, further proving that BMI is not a better predictor of body fat levels. Body adiposity index was high in 43% (42% in females and 45% in males) and very high in 24% (11% in females and 50% in the males). Body adiposity index (BAI) was found to be a good adiposity predictor

for metabolic syndrome which overcomes the limitations of BMI (4). Further, BAI values are comparable with the body fat percentage values obtained by more accurate dual-energy X-ray absorptiometry (DXA). Therefore, based on BAI more than 65% of the students are at risk of metabolic disorders in their later life.

Visceral fat is the most undesirable fat as it is more mobile and atherogenic compared to lower body fat. However, the mean visceral fat level was 4 ± 1 (3 ± 1 in females and 5 ± 3 in males) and only 2.3% (1% in females and 5% in males) of the population had high visceral fat while all of them were obese. The normal visceral fat levels even with overweight and obesity could be attributed to the young age of this population. Furthermore, 28% had high waist-to-height ratio and abdominal obesity in 24%, this could be due to high levels of subcutaneous fat in the abdomen. However, due to the redistribution of fat with age, which will increase the amount in visceral compartment, might increase their risk for CVD in their later life.

In the present population, BMI had very strong positive correlation with visceral fat level ($r=0.920$, $p<0.0001$) and a moderately strong correlation with TBF% ($r= 0.419$, $p<0.0001$). Visceral fat rate had strong correlations with WC ($r=0.886$, $p<0.0001$) and waist-to-height ratio ($r=0.826$, $p<0.0001$) while moderate positive correlation with WHR ($r=0.528$, $p<0.0001$).

A study conducted in a group of female undergraduates of University of Peradeniya , Sri Lanka, using a BIA system, revealed that the mean TBF% was 30 ± 9% while 22% and 18% of them had high and very-high body fat levels respectively (5). However, none of the participant had high visceral fat levels. These finding were comparable with the present study as female undergraduates in the present study had mean TBF% of 29 ± 5% while, 36% and 15% had high and very high TBF% and only one female student had high visceral fat level.

**Conclusions and Recommendations**

Based on waist-to-height ratio 28% and on BAI 65% of the undergraduates are susceptible for metabolic disorders in their later life, even though they were not in the obese category according to the BMI value. Therefore, they should be encouraged for long term behavioral improvement on diet and physical activity.

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# CUT OFF FOR HYPOVITAMINOSIS D BASED ON PARATHYROID HORMONE LEVELS AMONG A POPULATION OF PREGNANT MOTHERS IN COLOMBO DISTRICT

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## Introduction

Countries near to equator receive more sunlight throughout the year. However, sun-seeking behavior is uncommon in these populations due to hot climate. Epidemiologic studies from our neighboring country, India, have shown high prevalence (70%) of low vitamin D (< 20 ng/mL) in all age groups; neonates, preschool and school children, pregnant women and adult males. Rodrigo *et al.,* has reported 56% of premenopausal women having vitamin D level ≤14ng/mL, in Southern coastal belt of Sri Lanka [1]. In recent years, another study has shown vitamin D level of <20 ng/mL among 35% preschoolers[2].

Lack of a standard definition for optimal level and lack of consensus on the cut-off values for vitamin D levels has led to the terminologies; sufficiency, insufficiency and deficeincy. Further, comparison of published data on vitamin D status among diverse populations and regions has become difficult due to different reference values and methods that have been used to assess vitamin D. Hence, standardising the measurements will benefit policies on supplementation and food fortification programmes. Importantly, such policies or guidelines should consider national and cultural variations and food availability. Thus, this present study was undertaken to determine the cut off for hypovitaminosis D based on parathyroid hormone (PTH) levels among a population of pregnant mothers in the Colombo District.

## Materials and Methods

The study was carried out as a descriptive analytical study among a population of pregnant mothers (28 weeks to 40 weeks) from all the MOH areas in the Colombo district. The inclusion criteria were, uncomplicated pregnancy, single intrauterine pregnancy, gestational age 28 weeks and above and mothers who permanently reside in the Colombo district. The sample was selected to represent the pregnant mothers in the Colombo district. The study was carried out with statistically analysed sample number of 393 pregant mothers to describe the prevalence of vitamin D deficieny in the Colombo District. Sampling

was carried out as a stratified random sampling method. According to the population basis, samples were recruited from each MOH area. However, the Colombo Municipal Council (CMC) area is providing the calcium supplementation along with vitamin D as a product called "Kalzana". Thus, all the 6 MOH areas functioning under CMC were not considered in the sample recruitment as the vitamin D supplementation is one of the exclusion criteria for the recruitment process.

A pre tested interwiewer administered questionnaire was used in data collection. A venous blood sample was collected for the analysis of vitamin D, PTH, serum calcium, inorganic phosphorous and alkaline phosphatase (ALP).

The following laboratory methods were used in the sample analysis

- Serum vitamin D: The LIAISON 25 OH Vitamin D TOTAL assay kit
- PTH: The LIAISON N-TACT PTH Gen II assay kit
- Calcium, inorganic phosphorous and ALP: colorimetric method using Konealab analyzer

Participant's outdoor activities over the previous week in terms of duration (in minutes) and frequency (per week) were recorded in the questionnaire and the sun exposure (hours/week) was calculated.

Statistical analysis was performed using SPSS (version 15.0) software package. Correlation analysis and regression analysis were carried out to find out the relationship between serum vitamin D and other biochemical parameters. The Receiver operating curves (ROC) were used to find out the possible and suitable cut off for hypovitaminosis D based on PTH values.

**Results and Discussion**

Mean age of the maternal population was 29±6 years. Majority of the mothers were Sinhalese (75.3%), house wives (76.6%) and had secondary education (76.8%). The mean gestational age at birth was 32.9±3.5 weeks. The biochemical parameters of the study population are given in the Table 1.

**Table 1.** Biochemical parameters of the population

| Blood biochemical Parameters | Blood biochemical parameters | |
|---|---|---|
| | **Mean ± SD** | **Median (IQR)** |
| Corrected calcium (mmol/L) | 2.37±0.20 | 2.38 (2.46-2.32) |
| Inorganic phosphorous (mmol/L) | 1.20±0.15 | 1.21 (1.31-1.09) |
| Total ALP (IU/L) | 151.4±66.6 | 136.0 (181.3-105.8) |
| Bone specific ALP (IU/L) | 79.2±27.5 | 74.0 (95.0-62.0) |
| PTH (pg/mL) | 26.5±11.5 | 23.8 (31.1-18.4) |
| Vitamin D (ng/mL) | 18.63±7.55 | 17.9 (22.9-13.1) |

SD: Standard deviation; IQR: Inter quartile range

Serum inorganic phosphorus was within the normal range for the population. However, the corrected calcium was found to be low (normal 2.15- 2.57 mmol/L) among 07 mothers (1.8%). Total ALP (heat labile & heat stable) was above the cut off (>240 IU/L) in 10.2% (n=40). PTH levels above the cut off (>66.5 pg/mL) was observed only among 0.8% (n=03) of the population. Correlation analysis was carried out to find the association between serum vitamin D and other biochemical parameters. Only the serum corrected calcium (r=0.102;p=0.044), inorganic phosphorous (r=0.165;p=0.001) and PTH (r=-0.220;p=0.000) had a significant correlation with serum vitamin D levels. Then the regression analysis was performed to eliminate the effect of confounding factors such as age of the mother, occupation, monthly income, gestational age, and BMI. PTH levels and amount of sunlight exposure only had a significant association to serum vitamin D levels [3, 4].

The ROC was performed and corresponding area under the curve (AUC) was calculated. The figure 01 shows the ROC curve for the serum vitamin D levels based on the manufacturers recommendation for PTH cut off levels 66.5 pg/mL. AUC for the serum vitamin D levels was 0.933 at the level of 95% CI (p=0.010). Newer cut off value proposed to detect the hypovitaminosis D with highest accuracy, sensitivity (96.1%) and specificity (66.7%) is 8.1 ng/mL. This value is quite lower than the Institute of Medicine (IOM) cut off values. Prevalence of hypovitaminosis D according to newer cut off among pregnant mothers in the Colombo District is 4.1%. (n=16). Similar analysis had been carried out in another study from North India and had derived higher cut off value (22.5 ng/mL)[5]

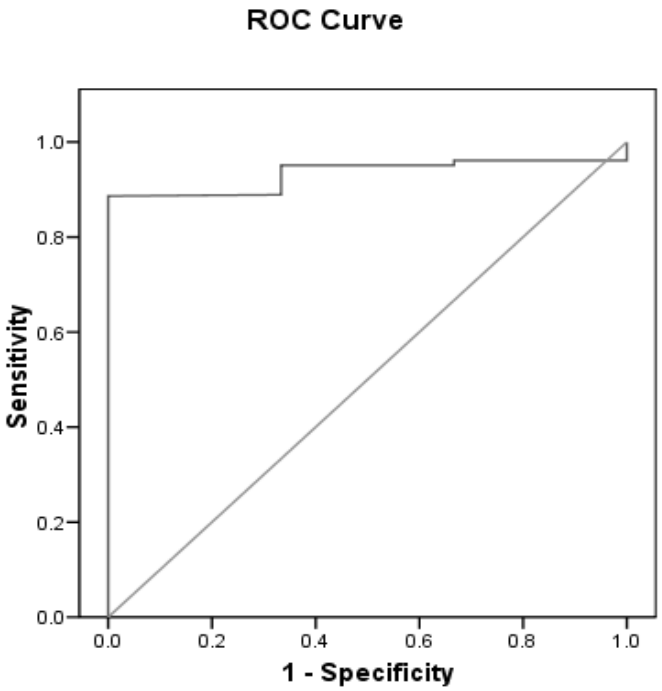


**Figure 1.** ROC for serum vitamin D levels based on PTH

**Conclusions and Recommendations**

The ROC curve between serum vitamin D and PTH yielded a cut of for vitamin D value, which is less than the cut off value recommended by IOM (20 ng/mL). There were no reported studies in Sri Lanka on serum vitamin D with bone biochemical, diet and sunlight exposure among pregnant mother. A larger sample size would have been more useful in finding out the cut off for hypovitaminosis D among a representative sample of Sri Lankan population. Larger randomized control trials are needed to investigate the need of vitamin D supplementation and safe dose.

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# EVALUATION OF HEPATOTOXICITY OF "*SINNA SIVAPPU MAATHIRAI*": A SIDDHA HERBO MINERAL DRUG, IN PATIENTS WITH RESPIRATORY DISEASES WHO VISIT SIDDHA TEACHING HOSPITAL KAITHADY, JAFFNA

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## Introduction

Siddha medical system has been practiced by Tamil speaking community in South part of India and North East part of Sri Lanka. *Sinna Sivappu Maathirai* (SSM) is a compound herbo-mineral Siddha drug. It is prepared by nine raw materials of herbs, one salt as Borax/ Sodium Bi Borate and a mineral, red sulphide of mercury. SSM is currently prescribed for cough;, breathing difficulties, cough with fever and chest pain while cough. "Ayurveda and Siddha products offer very little data on toxicological profile and more stringent toxicological requirements of these product as required by prominent regulatory agencies are still unmet" (1). A discussion on the use of heavy metals in herbal formulations and their safety was initiated by WHO with contribution of the regional experts of the field. Many participants of the discussion felt that any change of formulation or indication of a traditional medicine required safety studies, even if manufactured according to classical texts. So far, limited studies have been published on herbo-mineral preparations using in Sri Lankan Siddha medical system. According to the information, large quantity of SSM used widely per annum in Northern Provincial hospitals and Siddha teaching hospital since 2006. However, no report is available on safety evaluation of usage of SSM. Hence the present study designed to evaluate the hepatotoxicity of SSM in patients.

## Materials and Methods

*Study Drug – Sinna Sivappu Maathirai*

The drug is prepared as *Kuntriyalavu* according to the standard protocol in classical text of *Suthesa Vaithya Oudatha Thiraddu*. The pharmacy in Siddha teaching hospital Kaithady, prepare SSM accordingly and it is using to treat patients in the hospital. The drug has been prescribed as two doses of *Kuntriyalavu* two times a day after meals with *anupanm* of 5 ml betel extract. Siddha texts (2) mentioned that one *Kuntriyalavu* is equivalent to 130mg. The SSM is prescribed for the duration of 7 days. All raw materials including *Sathilingam (Redsulphide of Mercury)* used to prepare SSM are authenticated

and supplied by technical evaluation committee of the department of Ayurveda.

It was a descriptive observational study. Study carried out at Siddha Teaching Hospital, Kaithady, Jaffna, Sri Lanka. Study participants were patients prescribed with SSM for the treatment of respiratory symptoms (the patients treated for cough with phlegm, chest pain while cough and breathing difficulty) at Siddha Teaching Hospital, Kaithady. Patients who prescribed SSM for the first time included for the study. Children below 12years, pregnant women, and mentally incompetent patients, patients with hepatic and renal impairment were excluded.

According to Stephen *et al.* (2007) sample size was calculated. Maximum sample was 64. Structured interviewer administered questionnaire and investigation were performed by the researcher in the laboratory at Department of Biochemistry, Faculty of Medicine, University of Jaffna. Aspartate amino transferase (AST) and alanine amino transferase (ALT), bilirubin and ALP *(Alkaline Phosphatase)* were investigated to evaluate the hepato-toxicity. Pretest was done in 05 patients who presented to the Rural Siddha hospital Kodikamam for validation of the questionnaire. These patients were not included in the main study. After obtaining written informed consent, patients were recruited for the study till the desired sample size reached (Purposive sampling). Ethical clearance was obtained from Ethical Review Committee, Faculty of Medicine University of Jaffna Sri Lanka.

*Data collection*

After obtaining consent, the patients were recruited for the study and the detail medical history of the selected patients was taken through the structured interviewer administered questionnaire. All the patients were assessed at baseline and after 1$^{st}$ week, 2$^{nd}$ week, 4$^{th}$ week and 12$^{th}$ week after completing one week treatment. All the data collection was performed at OPD of Siddha Teaching Hospital Kaithady.

*Collection of blood*

Before collecting blood, the participants were informed and obtained written consent for withdrawing blood. Nursing officer from Siddha Teaching Hospital Kaithady collected blood. The laboratory at Siddha Teaching Hospital was used with the permission of Medical officer in-charge of the hospital. Blood samples (5 ml from each patient) were collected by venipuncture and stored in appropriate containers. Each blood sample was given an identity number based on the serial number of questionnaire and data record. Samples transported to Department of Biochemistry Faculty Medicine, University of Jaffna in an ice box ($4^0$c) within 4 hours and lab procedures continued. After completion of Laboratory investigation, the sample disposed by incineration.

*Data analysis*

Mean, standard deviation, percentile paired T-test and correlation of coefficient were calculated using SPSS 22.

## Result and Discussion

**Table 1.** Gender based AST (U/L) distribution, mean difference (MD) and its significant level

| Assessment | AST (U/L) - Male (41) | | | | | | | AST (U/L) - Female (23) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | ± | SD | MD | ± | SD | Sig. | Mean | ± | SD | MD | ± | SD | Sig. |
| BL | 23.18 | ± | 9.7 | | | | | 19.3 | ± | 1.29 | | | | |
| FW1 | 23.76 | ± | 9.7 | -0.6 | ± | 5.95 | 0.53 | 18.61 | ± | 1.19 | 0.7 | ± | 1.81 | 0.08 |
| FW2 | 23.27 | ± | 9.5 | -0.1 | ± | 6.54 | 0.93 | 18.25 | ± | 1.09 | 1.06 | ± | 3.49 | 0.16 |
| FW3 | 23.59 | ± | 12 | -0.4 | ± | 8.97 | 0.77 | 18.29 | ± | 1.31 | 1.01 | ± | 6.69 | 0.47 |
| FW4 | 22.33 | ± | 9.9 | 0.85 | ± | 8.53 | 0.53 | 19.46 | ± | 1.85 | -0.16 | ± | 9.77 | 0.94 |

Cut-of value of AST (>37 U/L for male and >31 U/L for female) (3), SD – Standard Deviation; B/L – Baseline, FW1 - Follow up 1(Completion of first week), FW2 - Follow up 2 (Completion of second week),

AST values observed in the subjects at baseline and follow ups were in normal range according to the standard reference. MD (Mean difference) between base line and follow-up levels of were statistically not significant in males as well females. Same as in the standard reference, gender based distribution of AST was lower in females than males.

**Table 2.** Gender based ALT (U/L) distribution & mean difference and it's significant

| Assessment | ALT (U/L) - Male (41) | | | | | | | ALT (U/L) - Female (23) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | ± | SD | MD | ± | SD | Sig. | Mean | ± | SD | MD | ± | SD | Sig. |
| BL | 28.28 | ± | 16.81 | | | | | 14.16 | ± | 9.77 | | | | |
| FW1 | 30.24 | ± | 19.18 | -1.97 | ± | 10.1 | 0.22 | 14.01 | ± | 8.69 | 0.14 | ± | 3.69 | 0.85 |
| FW2 | 29.06 | ± | 16.99 | -0.78 | ± | 6.69 | 0.46 | 13.97 | ± | 7.95 | 0.19 | ± | 5.39 | 0.87 |
| FW3 | 29.69 | ± | 21.00 | -1.41 | ± | 11.3 | 0.43 | 13.38 | ± | 4.84 | 0.78 | ± | 7.68 | 0.63 |
| FW4 | 27.83 | ± | 18.15 | 0.45 | ± | 9.41 | 0.76 | 14.39 | ± | 7.31 | -0.2 | ± | 4.81 | 0.82 |

Cut-of value of ALT (>40 U/L for male and >31 U/L for female) (3)

ALT values observed in the subjects at baseline and follow ups were in normal range according to the standard reference. MD (Mean difference) between base line and follow-up levels of were statistically not significant in males as well females. Same as in the standard reference, gender based distribution of ALT was lower in females than males.

**Table 3.** Bilirubin (mg/dl) level between base line and follow-ups in different age categories

| Age Range (years) | Bilirubin levels (mg/dl) in the baseline and follow-ups | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BL | | | | FW1 | | | FW2 | | | FW3 | | | FW4 | | | | |
| | N | Mean | ± | SD | Mean | ± | SD | Mean | ± | SD | Mean | ± | SD | Mean | ± | SD | | |
| 18-28 | 19 | 1.44 | ± | 0.87 | 1.40 | ± | 0.54 | 1.31 | ± | 0.48 | 1.19 | ± | 0.5 | 1.23 | ± | 0.48 | | |
| 29-38 | 16 | 1.38 | ± | 0.55 | 1.36 | ± | 0.51 | 1.43 | ± | 0.56 | 1.34 | ± | 0.4 | 1.16 | ± | 0.38 | | |
| 39-48 | 16 | 1.18 | ± | 0.99 | 0.71 | ± | 0.15 | 1.00 | ± | 0.65 | 0.87 | ± | 0.3 | 0.80 | ± | 0.14 | | |
| 49-58 | 8 | 1.55 | ± | 1.01 | 1.25 | ± | 0.79 | 1.30 | ± | 0.78 | 1.22 | ± | 0.7 | 1.08 | ± | 0.44 | | |
| 59-68 | 12 | 0.77 | ± | 0.27 | 0.79 | ± | 0.27 | 0.78 | ± | 0.29 | 0.86 | ± | 0.3 | 0.85 | ± | 0.23 | | |
| 69-78 | 1 | 2.03 | ± | 0.00 | 1.47 | ± | 0.00 | 1.50 | ± | 0.00 | 1.5 | ± | 0 | 2.17 | ± | 0 | | |
| **≥ 18** | **64** | **1.29** | **±** | **2.78** | **1.17** | **±** | **0.56** | **1.20** | **±** | **0.57** | **1.13** | **±** | **0.5** | **1.08** | **±** | **0.42** | | |

Reference value Adult: 0.17-1 mg/dl (4)

**Table 4.** Mean difference and its Significant Bilirubin level (mg/dl) between baseline and follow-ups

| | Bilirubin (mg/dl) | | | |
|---|---|---|---|---|
| **Assessments** | **MD** | **±** | **SD** | **Sig.** |
| BL-FW1 | 0.12 | ± | 0.77 | 0.22 |
| BL-FW2 | 0.09 | ± | 0.72 | 0.33 |
| BL-FW3 | 0.16 | ± | 0.69 | 0.07 |
| BL-FW4 | 0.21 | ± | 0.72 | 0.02 |

Serum bilirubin concentration in the subjects at the baseline as well as follow ups were higher than normal range according to the standard reference. There was no significant difference (P-value < 0.05) between the mean difference between baseline and follow ups except 4th follow-up.

**Table 5.** ALP (IU/L) level between baseline and follow-ups in different age

| Age Group | ALP levels (IU/L) at baseline and follow-ups | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BL | | | | FW1 | | | FW2 | | | FW3 | | | FW4 | | | | |
| | N | Mean | | SD | Mean | | SD | Mean | | SD | Mean | | SD | Mean | | SD | | |
| 18-28 | 19 | 175.54 | ± | 43.34 | 167.47 | ± | 40.83 | 168.0 | ± | 38.86 | 171.18 | ± | 39.6 | 167.00 | ± | 39.48 | | |
| 29-38 | 16 | 161.54 | ± | 40.83 | 160.65 | ± | 38.34 | 162.2 | ± | 44.33 | 161.31 | ± | 39.78 | 152.85 | ± | 28.04 | | |
| 39-48 | 16 | 143.54 | ± | 23.29 | 158.83 | ± | 16.55 | 149.7 | ± | 17.77 | 152.38 | ± | 20.47 | 160.79 | ± | 19.01 | | |
| 49-58 | 8 | 176.63 | ± | 37.78 | 175.29 | ± | 33.10 | 172.9 | ± | 26.5 | 168.92 | ± | 21.56 | 179.25 | ± | 35.25 | | |
| 59-68 | 12 | 176.89 | ± | 44.13 | 177.42 | ± | 39.61 | 178.8 | ± | 40.36 | 176.31 | ± | 44.22 | 180.97 | ± | 39.72 | | |
| 69-78 | 1 | 207.00 | ± | 00.00 | 207.00 | ± | 00.00 | 204.0 | ± | 00.00 | 182.00 | ± | 00.00 | 217.00 | ± | 00.00 | | |
| **≥ 18** | **64** | **168.92** | **±** | **40.55** | **168.15** | **±** | **36.39** | **167.47** | **±** | **37.33** | **167.21** | **±** | **36.41** | **167.62** | **±** | **35.18** | | |

ALP range: 98-279 IU/L (5)

**Table 6.** Mean difference and its Significant of ALP (IU/L) Between baseline and follow-ups

| ALP (IU/L) | | | | |
|---|---|---|---|---|
| Assessments | MD | ± | SD | Sig. |
| BL-FW1 | 0.78 | ± | 18.6 | 0.74 |
| BL-FW2 | 1.45 | ± | 19.4 | 0.55 |
| BL-FW3 | 1.71 | ± | 19.4 | 0.48 |
| BL-FW4 | 1.30 | ± | 24.1 | 0.67 |

ALP values observed in the subjects at baseline and follow ups were in normal range according to the standard reference. In this study, same as in the standard reference, aged based distribution of ALP was lower in 18 and above years. MD (Mean difference) between base line and follow-up levels of were statistically not significant among the age groups. The result showed that the AST, ALT, ALP and bilirubin were not significantly increased by using SSM for one week continuously.

**Conclusion and Recommendation**

According to the results, one week treatment of SSM showed no hepatotoxicity in treated patients. Therefore, SSM can be recommended to use for a week period to treat respiratory symptoms in adults above 18 years old.

**Acknowledgement**

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# FOCUS AREA

## Information Communication Technology & Knowledge Services

# TEACHER PERCEPTION ON ICT USE IN THE CLASS ROOMS: A CASE STUDY IN KALUTARA DISTRICT

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## Introduction

Information Communication Technology (ICT) is defined as a "diverse set of technological tools and resources used to communicate and to create, disseminate, store and manage information [1]. Many countries in the world, have introduced ICT into schools via different courses of action.

Teachers play an important role in integrating ICTs in the classroom. Without the involvement of teachers, most students may not take advantage of all the available potential benefits of ICT on their own. Teachers need to actively participate in using ICTs. The tendency of using ICT in teaching and learning may strongly affect by the attitudes of the teachers.

This study aims to investigate teachers' perceptions towards ICT integration in the teaching- learning process. This was based on the assumption that the successful use of ICT is helpful to motivate the students in the teaching-learning process and enhance the quality of education.

## Methodology

The population for this study was all national school Science, Mathes, English teachers who teach grades 9-11 in Kaluthara District. Kaluthara District has 20 national schools in three education regions namely Kaluthara, Horana, and Mathugama. 8 schools were selected for the study using cluster sampling. A total of 80 questionnaires were distributed among participants and 73 questionnaires were received from the participants. Data, in this study, were collected through self-administered questionnaires which included both open ended and closed-ended questions. For the analysis of collected data, both qualitative and quantitative methods were used. Descriptive methods and Statistical Packages for Social Sciences (SPSS) software are used to analyze the data.

## Results and Discussion

Respondents consisted of 34% of male teachers and 66% of female teachers who teach Science, English and Mathematics subjects in national schools located in Kalutara District. Daily ICT usage of school teachers was found to

be as follows, 41.1% of teachers use ICTs less than one hour, 26.0% of teachers use ICTs between 1-3 hours, 20.55% of teachers use a between 3-5 hours and 12.33% of teachers use more than five hours a day. This says all the respondents use ICTs at least 1 hour per day in school hours or at home. In this study, including one item, that was related to the respondents familiarity with ICT. When the respondents were asked about their personal experience with ICT, it was found that the majority of teachers (37%) had more than 5 years of experiences in using ICTs. This belief is a clear indication of the school teacher's familiarity with ICT. Although, this does not necessarily mean that those teachers integrate ICT into the subjects. 23% of the respondents were less than one year users of ICT as it can be seen in figure 1.

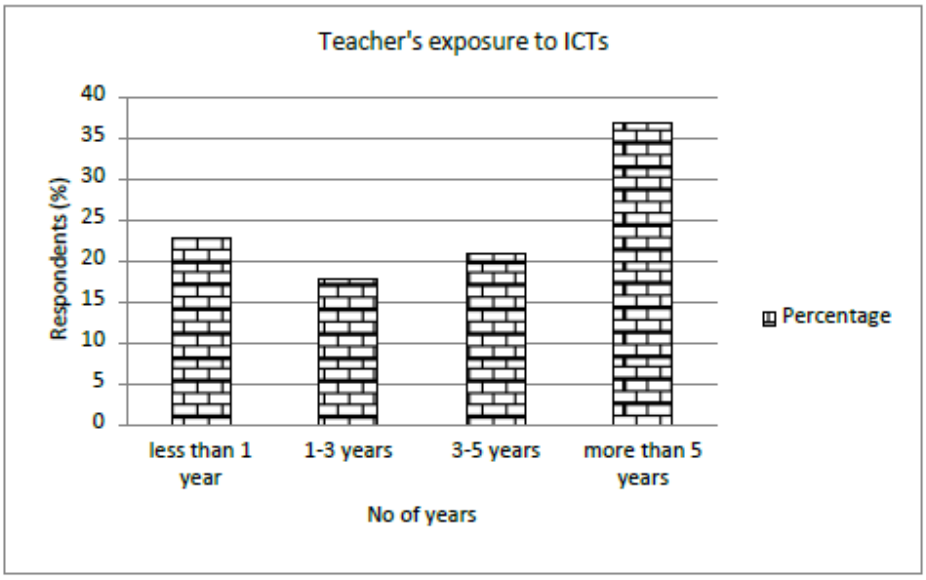


**Figure 1.** Distribution of the respondents by their exposure to ICT

The actual usage level of ICT in the classroom is 37.0% of respondents specified as high and 63.0% of respondents specified as low. When considering computer software used in the classroom MS Word, PowerPoint, Web browsers, and E-mail were used more frequently (e.g. Daily or weekly basis) compared with other software (Table 1). Most of the respondents are unable to use MS Access and Newsgroups due to the skill gap. The percentage of respondents who can use search engines is lower than those who can't use it. The reasons mentioned by the teachers were unavailability of network expansion of the school, less no of computers in schools. The majority of the participants are unable to use computer software in the classroom due to mainly shortage of resources. This indicates the majority of teachers are not integrating ICT in the classroom.

**Table 1.** Distribution of respondents by the frequency of using computer software in the classroom

| | Usage | | | |
|---|---|---|---|---|
| Computer Software | Daily | Weekly | Sometimes | Never |
| | (%) | (%) | (%) | (%) |
| MS Word | 32.88 | 8.22 | 30.14 | 28.77 |
| MS Excel | 6.85 | 12.33 | 41.10 | 39.73 |
| Power Point | 13.70 | 9.59 | 58.90 | 17.81 |
| MS Access | 0 | 1.37 | 15.07 | 83.56 |
| Web Browser | 28.77 | 19.18 | 39.73 | 12.33 |
| Search Engine | 5.14 | 8.22 | 59.32 | 32.33 |
| E-mail | 15.07 | 28.77 | 45.21 | 12.33 |
| Newsgroups | 0 | 1.37 | 21.92 | 76.71 |

*The relationship between perception of teachers and ICT usage level of teachers*

According to the results, the teachers have a positive perception of the ICT usage in the classroom. Respondents strongly believe that the usage of ICT in the classroom increases the student's interest in learning. Correlation analysis was conducted to determine if there is any relationship between teacher perception on ICT integration and ICT usage level. The results indicated that there is no relationship between the teacher's perception of ICT integration and ICT usage level.

*The relationship between barriers to ICT integration and ICT usage level of teachers*

Some of the major barriers in using ICTs in classroom, as identified by the respondents, are time is not adequate to prepare materials based on technology (70.9%), lack of facilities in school (77.8%), deficiency in professional development opportunities for gaining knowledge and skill (87.6%), inadequate technical support for ICT integration (79.5%) and the inadequate of teachers' technical knowledge to prepare materials based on technology 72.6%). Correlation analysis was conducted to determine if there any relationship between barriers to ICT integration and ICT usage level. The results indicates that there is a significant ($\alpha$=0.05) relationship between barriers to ICT integration and ICT usage level ($r$=0.286, $p$= 0.032).

**Conclusion and Recommendation**

Respondents have a positive perception of ICT integration in the classroom. However, this does not certainly mean that they integrate ICT into the classroom. Also, lack of technical support services, lack of teacher training opportunities, lack of resources, the inefficiency of technical knowledge on ICT integration are the significant barriers integrate ICT in the classroom.

Age, Education qualification, ICT experiences are significantly affected by the actual use of ICT in the classroom. Regarding the use of instructional tools and materials in the classroom, the data shows that printed materials, Television/Video, Internet were the most frequently used by teachers in the classroom. The study concludes that the respondents had favorable perceptions towards using ICTs in the classroom and they need support from education authorities to improve their ICT skills. Further, it is necessary to provide technical support to facilitate ICT integrations in the classroom.

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# COMPUTATIONAL MODEL FOR DETECTING GRAMMATICAL MISTAKES IN SINHALA TEXT

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## Introduction

Sinhala being, the language spoken by about 16 million Sinhalese people in Sri Lanka, where it is one of the officials and the national languages, along with Tamil, greater part of the exercises at government experts, services and offices are completed in Sinhala whereas a large portion of the general population doesn't have a clue on how to utilize language [1]. It is essential to utilize the language accurately so as to safeguard the planned significance where the normal significance can be distinctive because of the inaccuracy of grammar. When thinking about the composed setting, Sinhala is an intricate language that contains many spelling and syntax rules where the rightness of the formal composing thoroughly relies upon these well-characterized rules [1]. It is imperative to guarantee the spelling and syntactic accuracy to convey the ideal significance from the perspective of automated materials with the unavailability of resources even though there are enough amount of available materials as hard copy and books for the Sinhala language. On the other hand, with the high multifaceted nature of the language, it sets aside extensive effort to physically edit the substance of a composed setting. The necessity of an automated system to play out this assignment has risen for the Sinhala language numerous years back. Well-created grammar checking applications are accessible for the dialects like English Tamil and Chinese and for plenty of many other languages [2]. But a morphologically rich language like Sinhala lacks precise grammar checking tools mainly because of the unavailability of enough resources [3]. Natural languages like Sinhala are not constrained by a specific syntax and have a wide range of vocabulary; hence, Sinhala grammar checkers must also have an extensive dictionary of most words with their complete meanings and part-of-speech usage [1]. Thus, this study was conducted in order to address the knowledge gap in detecting and correcting grammatical mistakes in Sinhala written text with the help of possibly available and publically accessible useful resources while providing a novel approach aligned with the neural network.

## Materials and Methods

This study has focused on detecting the grammatical mistakes in simple active sentences in Sinhala text where sentences in written text format were checked sentence-wise and word-wise respectively and the nouns and verbs were separately analyzed with the help of a resourceful part-of-speech (pos) tagger

and a morphological analyzer. Finally, a system has been implemented which comprised of a hybrid novel approach combining a rule-based system and a neural network system. Specifically, here we have considered only the already spell checked sentences for the detection for grammatical mistakes which helped compress the scope to a feasible level and provide more accuracy for the study. The main approach which has been proposed and implemented in this study is as follows,

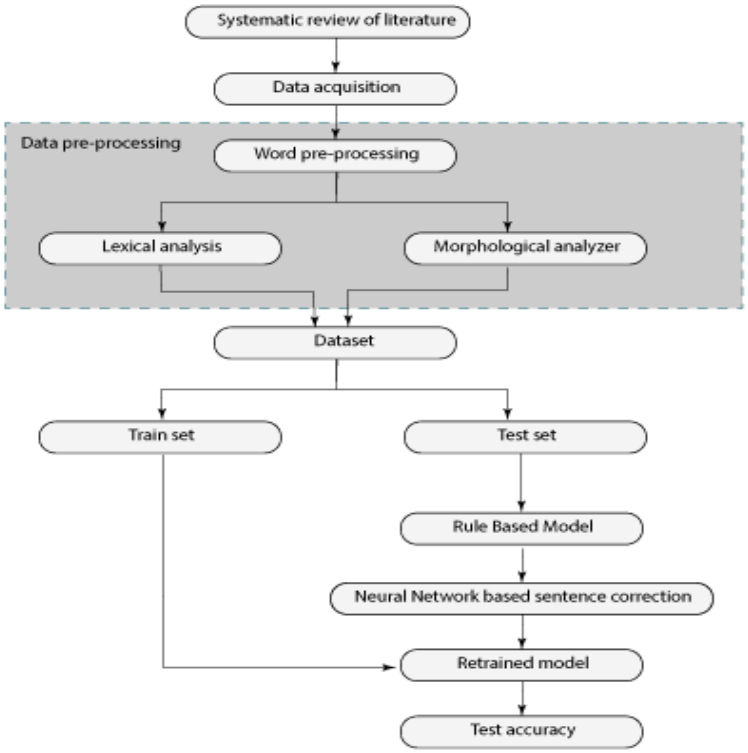


**Figure 1**. Flow chart depicting the proposed and implemented methodology for the study

*Data Acquisition*

About 250 three-word Sinhala simple active sentences were gathered manually from the web via Python scripting and they were categorized according to their tenses and grammatical person. Through this, a noisy web data set was created. Also, the Sinhala words required for the execution of algorithms required a Sinhala WordNet to find synonyms. Due to the lack of a fully functional and publically accessible WordNet for the Sinhala language, we have used a sample database of Sinhala words with their synonyms instead [2].

*Data Pre-processing*

Separation of sentences in the paragraph, separation of words from spaces in a sentence, removing unwanted characters from the sentences was been carried out in order to pre-process the data input into the system. Then the identification of subject and verb through the already pre-processed sentences were been carried out using an existing pos tag system and the tag of each word was identified and stored separately with the corresponding word for further reference [1]. A comprehensive, multi-level pos tag set for Sinhala was being used here which has been developed by the Center for National Language Processing, University of Moratuwa while considering its accuracy level. Then a morphological analyzer was used to separate the root and the suffix from the identified verb and separated root and suffix of the verb was further carried over to the next phase with the corresponding noun acquired from the above lexical analysis. Due to lack of access to most of the accurate morphological analyzers for Sinhala [3], on the notion that the above mentioned morphological analyzers work, we have used another existing considerably accurate morphological analyzer which is commonly known as “polyglot” in this study which is a natural language pipeline that supports Sinhala language in this study [5].

*Hybrid approach with rule-based and neural network*

The system has used 22 predefined sets of grammar rules of lexicons. Accordingly, for grammatically incorrect sentences, the system has predicted suggestions using a neural network algorithm [4] for all three tenses. Then the suffix-engine would try to concatenate given suffix and root of the verb and create a meaningful sentence to the user while finally providing the user with a correctly spelled and grammatically sound sentence.

*Testing accuracy*

In order to evaluate the performance and accuracy of the proposed method, we have used a set of several sample test cases. The simple Sinhala active sentences with no spelling errors with a word range of 3-5 words from the online edition of Sinhala newspapers was been selected for this purpose. Spelling check has already been done prior to testing of grammatical mistakes with the help of existing accurate spell checker for the Sinhala language; “Sinhala Spell Checker | Apache Open Office Extensions”.

**Results and Discussion**

Once the sentence is entered into the system, first, each word was been assigned with a tag by the pos tagger.

Eg: - In the sentence, “මම ගමට ගියෙමි”, it worked as below.

- Pronoun -> මම Verb -> ගියෙමි
- Common Noun -> ගමට

| | subject | tense | person | gender | animate | number | verb_root | is_correct |
|---|---|---|---|---|---|---|---|---|
| 2 | මම | අතීත | උත්තම | නොමැත | ප්‍රාණවාචී | ඒක | එමි | 1 |
| 3 | මම | අතීත | උත්තම | නොමැත | ප්‍රාණවාචී | ඒක | ඒය | 0 |
| 4 | මම | අතීත | උත්තම | නොමැත | ප්‍රාණවාචී | ඒක | එමු | 0 |
| 5 | අපි | වර්තමාන | උත්තම | නොමැත | ප්‍රාණවාචී | බහු | අමු | 1 |
| 6 | අපි | වර්තමාන | උත්තම | නොමැත | ප්‍රාණවාචී | බහු | අමි | 0 |

**Figure 2**. Sample dataset used for the decision tree algorithm in the study

The array which consisted above details was then parsed to a parser to identify its sentence pattern. Currently, the parser has been trained to identify 5 given patterns. Then a decision tree-based algorithm has been used to evaluate the verb with the “subject” and output feedback about the correctness of the sentence. To train this decision tree we used a dataset of 200 records which included data about 22 predefined grammar rules in Sinhala. This model then gave us an accuracy score of 84.61%.

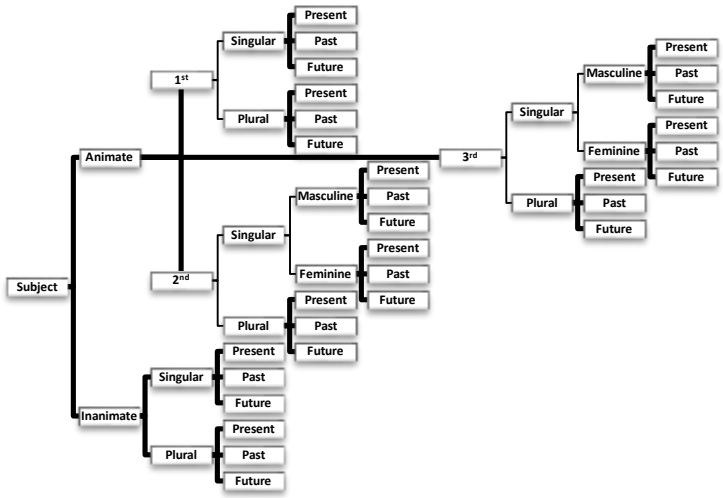


**Figure 3**. Grammatical rule-based decision tree of grammatical features used for grammatical error detection and correction in the study

If the grammar was incorrect, we have parsed the sentence to our neural network based sentence correction algorithm to suggest a correct sentence. It then suggested a suitable verb root and then added it to a matching verb suffix.

```
{
    "sentence": "කමල් සමග මම පාඩම් කරමි",
    "is_correct": false,
    "suggesions": {
        "present": "කමල් සමග මම පාඩම් කරමු",
        "future": "කමල් සමග මම පාඩම් කරන්නෙමු",
        "past": "කමල් සමග මම පාඩම් කළෙමි"
    }
}
```


**Figure 4**. Neural network based corrected grammar suggestion

If we consider the sentence, "මම ගෙදර කමු" here the word gives a meaning of "I eat the house", but it is not semantically correct. Our algorithm could escape this problem and it then suggested a more accurate word. So, this algorithm could be used to suggest a correct verb in any given word processing software.

**Conclusions and Recommendations**

In this research, we tried to introduce a hybrid approach for identifying grammatical errors and suggesting corrections for the Sinhala written sentences. With the above results, we were able to identify the sentence pattern in the given sentence and then identify its grammar rules. Further, using the neural network we were able to suggest corrections for the sentences identified as incorrect grammatical sentences with an acceptable accuracy rate according to the above results. Therefore with the results obtained from this study, we can conclude that the hybrid approach is an efficient and accurate approach for detecting and correcting grammatical mistakes in written text formats for the Sinhala language.

As suggestions for future work, the dataset must be developed further to identify more errors mostly in third person format and also the model should be optimized to identify more sentence patterns to perform well. Also for future implementations, it can be possibly recommended to use neural network systems [4] and try out mathematical model text classification in order to compare the accuracy of the hybrid system implemented in this research study which was developed by combining rule-based and neural network systems so as to improve the development of Sinhala language-based researches in the future.

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# FOCUS AREA

## Textile and Apparel

# THE IMPACT OF EMPLOYEE RELATIONSHIP MANAGEMENT ON EMPLOYEE PERFORMANCE IN APPAREL SECTOR: A STUDY IN ABC FIRM SRI LANKA

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## Introduction

Sri Lanka is one of the best apparel-producing countries in the world relative to its population. There are large numbers of private sector companies engaging in the apparel industry. Among them, ABC firm is one of the largest apparel exporters in Sri Lanka which is appealing in developing, manufacturing and marketing apparel products to globally expanded latest brands.

This study is focused on the impact of ERM in the apparel industry. Employee relationship management, which effectively monitors and manages the relationship between individuals of the same team or different teams is a special field in Human Resource Management. Employee relationship management activities within the organization, help in strengthening the bond among the employees and ensures that each one is satisfied and maintains a healthy relationship with each other. It includes various activities undertaken by the superiors or the management to develop a healthy relationship among the employees and to extract the best out of each team member ultimately while achieving the goals of the organization.

When achieving any kind of organizational objectives, there should be the best human resource management practices in line with better employee relationships for an organization. Employees' attitudinal factors for their job performance remain as positively correlated in nature if there are the best human resource management practices. This study aims to examine the effect of ERM on employee's performance at textile and apparel and provide recommendations and suggestions on how to apply ERM in the organizations

## Materials and Methods

*Materials*

Good employee relation motivates employees for better performance and contributes to the overall success of the organization [3]. They also conclude that employee relations management components such as communication, participative leadership, shared goals and value, mutual trust, motivation and conflict management have significant effect on the performance of employees.

Pradeep and Prabhu [1] reveal that there is a positive relationship between transformational leadership and employee performance as this style of

leadership creates conducive work environment, job satisfaction and extra effort in comparison to the counterpart transactional leaders and these leaders have the capacity to convince their subordinates to achieve more.

In a survey conducted by Bono and Judge [4] as to whether the followers of transformational leaders exhibit higher performance, motivation, job satisfaction, and organizational commitment in service and manufacturing organizations, it was found that Transformational Leadership behaviors, as evaluated by followers, was positively related to followers' job performance.

Judge and Piccolo [5] found that the Transformational Leadership had shown the highest overall validity, while contingent reward leadership was a close second. The authors found more validity with Transformational Leadership than contingent rewards when looking at 201 leader effectiveness.

Chaubey, Rajat and Navita [2] reveal that if Employees Relationship management practices i.e. Discipline, Conflict management, Trade Union, Communication, Employee Empowerment and Involvement and Encouragement of employee suggestion are implemented in organizations and managers give it high attention it can enhance ERM status in organizations and help employees in getting satisfaction from their job. Thus, it emphasizes on performance, growth and development of employees for creating competitive advantage.

*Method*

Direct employees who are currently working in the firms were selected in order to achieve the objectives of the study. Total sample size of the study was 300. Six branches were selected and 50 employees were selected from each branch. Primary Data was collected using a pre-tested structured questionnaire which was developed by using a five-point Likert scale. After collection of data, descriptive analysis was carried out as preliminary analysis to understand the situation properly. Correlation analysis was conducted in order to study the relationship between variables using Pearson's correlation coefficient. Then stepwise multiple regression analysis was used to identify the factors.

**Results and Discussion**

The result reveals that there are significant positive correlations between ERM components with Employee Performance. Corporate Communication and Trust indicate a moderate positive linear relationship with employee performance and HR Practices, Shared Goals/Values and Leadership Style indicate a strong positive linear relationship with employee performance.

From this paper it is agreed that ERM components has positive effect on the employees' performance in apparel sector. Organizations are realizing that ERM helps them to build stronger relationship with employees. As such ERM can

build and enhance relationships and to the company and improve the employees' performance.

*Results of the multiple linear regressions*

In multiple linear regressions, there are 5 independent variables, and the relationship between the dependent variable and the independent variables is represented by the following equation:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \alpha$$

Where, $Y$ = Employee Performance
$X_1$ = HR Practices
$X_2$ = Corporate Communication
$X_3$ = Trust
$X_4$ = Shared Goals and Values
$X_5$ = Leadership Style
$\alpha$ = constant

**Table 1.** Pearson Correlation Analysis

| | $Y$ | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X$ |
|---|---|---|---|---|---|---|
| Y | 1 | | | | | |
| $X_1$ | 0.894092081 | 1 | | | | |
| $X_2$ | 0.545503625 | 0.043615779 | 1 | | | |
| $X_3$ | 0.514024075 | 0.148587239 | 0.0906230646 | 1 | | |
| $X_4$ | 0.75368306 | 0.038022882 | 0.0863916923 | 0.12795434461 | 1 | |
| $X_5$ | 0.812236392 | -0.076573779 | -0.0807380423 | -0.0841279954 | -0.0069589609 | 1 |

**Table 2.** Model Summary

| **Regression Statistics** | |
|---|---|
| Multiple R | 0.976583914 |
| R Square | 0.963179497 |
| Adjusted R Square | 0.961555568 |
| Standard Error | 0.164924165 |
| Observations | 300 |

The value of R Square (0.963179497) and Multiple R (0.976583914) show that there is strong association between the independent variables and the dependent variable with the standard error of 0.16492416. In additions, the Table 2 implies that the Employee Performance of the ABC firm's employees is 96% dependent on HR Practices, Corporate Communication, Trust, Shared Goals/Values and Leadership Style

**Table 3.** ANOVA Test

| | df | SS | MS | F | Sig F |
|---|---|---|---|---|---|
| Regression | 5 | 12.01239415 | 2.40247883 | 611.5916597 | 5.39731E-22 |
| Residual | 294 | 1.154902564 | 0.00392824 | | |
| Total | 299 | 11.94083519 | | | |

The F value of the test for the data is 611.5916597. The p-value associated with this F value which is 5.39731E-22 which is lower than the alpha value 0.05. An examination of ANOVA table makes it clear that the five independent variables in the standard model are significantly predictive of the dependent variable.

**Table 4.** Regression Coefficients

| | Coefficients | Standard Error | t Stat | P-value |
|---|---|---|---|---|
| Intercept | 0.594141612 | 0.150122791 | 3.625514812 | 0.002372333 |
| HR Practices | 0.620157356 | 0.154437306 | 4.570986073 | 0.000065985 |
| Corporate Communication | 0.217463554 | 0.157606068 | 5.031540961 | 0.000346793 |
| Trust | 0.322626903 | 0.05321033 | 5.555205324 | 0.000173652 |
| Shared Goals and Values | 0.521803129 | 0.170456609 | 7.070514392 | 1.60543E-07 |
| Leadership Style | 0.531097141 | 0.070542654 | 7.614160174 | 0.000248873 |

The study includes the following hypothesis:

$H_0$: There is no significant relationship between ERM components and the employee's performance.
$H_1$: There is a significant relationship between ERM components and the employee's performance.

Since all p values < 0.05, the all null hypothesis can be rejected. Therefore, there is significance relationship between HR practices, communication, Trust, shared goals and values, leadership style with employee performance and constant also significant. The derived multiple regression model is,

$$Y = 0.620157356X_1 + 0.217463554X_2 + 0.322626903X_3 + 0.521803129X_4 + 0.531097141X_5 + 0.594141612$$

**Conclusions and Recommendations**

Maintaining healthy employee relationship among different level of employees within an organization is essential for steady productivity and the goal/target achievement of any kind of organization. Anyhow most of the time apparel sector is difficult to maintain healthy employee relationship due to the unique culture associate with that industry. The main purpose of this study is to investigate about Employee Relationship Management (ERM) on employee performance. According to this research, ERM components have positive effect

on the employees' performance in Apparel field and it can bring lots of benefits to the organization. As overall this study becomes an evident that ERM can build and enhance relationships and strengthen commitment to the firm and improve the employees' performance. ERM components are ordered according to their effect on the employees' performance where noticed that HR practices has the biggest effect according to the analysis, and then Leadership Style, Shared goals/values, Trust and finally the Corporate Communication. The final results show that ERM components significantly impact on the performances of employees in positive way. For the future success of the organization, it is necessary to have good employee relations in order to maintain good employee performance.

Based on the findings, it is important to implement practicable strategies to reduce the disagreement level on particular ERM practices and improve the significant level of the ERM practices to the performance of employees. That it is to pay attention to all components of ERM involving HR practices, communication, trust, leader ship styles and shared goals and values as important variables because they can improve employee performance on long term. Further consideration on training offered by the firm, it needs to be equipped with necessary information, techniques and skills, which facilitate the capability of job completion in the field of particular employee and eventually help in improving their performance should improve communication within the employees through interchange ideas, feelings and opinions with management to strength the relationship with employees and should clarify goals and values, provide formal and informal feedback, and engage employees in open and honest dialogue, so they can improve the relationships they share with employees.

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