8TH YSF SYMPOSIUM

January 30, 2019

Organized by

Young Scientists Forum

National Science and Technology Commission

ISBN: 978-955-8630-12-9

Published by:

National Science and Technology Commission,

No.31/9,31/10, Dudley Senanayake Mawatha,

Colombo 08.

www.nastec.lk

Table of Content

Massage from the Acting Director

National Science and Technology Commission

I am pleased to convey this message as the Director / CEO of National Science and Technology Commission (NASTEC) on the inauguration of the 8th Annual Research Symposium of the Young Scientists Forum (YSF) organized by the NASTEC in collaboration of the Young Scientists Forum (YSF).

According to my observations, since its inception in 2012, this symposium has been annually increased to a higher standard to give it to a due prestige.

It is my pleasure to see the progress of the young scientists facing the challenges with much dedication to initiate research from the planning stage to invent and innovate to enhance the economic development of Sri Lanka in contributing to improve the quality of life of our people. I presume that more and more immediate research and development interventions should take place to enhance the development of our country.

I hope that the young scientists who are actively participating in this event will play a major role to lead a glittering science culture in this country adding values to future leaders in science in Sri Lanka.

I wish good luck for the proceeding of the sessions and all future endeavors of YSF.

Eng. D.D. Ananda Namal

Act. Director/CEO
National Science and Technology Commission

Massage from the Steering Committee Chairman

Young Scientists Forum

It is with great pleasure that I welcome all of you to the 8th YSF Symposium organized by the Young Scientists Forum of the National Science and Technology Commission (NASTEC). This symposium is held annually from 2012 and provides an opportunity for the Young Scientists of Sri Lanka from different fields to present their research findings, interact with each other and develop research collaborations.

The symposium brings together researchers in the diverse fields of Agriculture, Food Science and Nutrition, Environmental Sciences, Engineering, Computer Science, Biochemistry & Chemistry, Medicine & Health Sciences, Economics, Social Sciences, Molecular Biology. Biotechnology and Molecular Biology. The 50 extended abstracts received for this year's symposium underwent a thorough peer review process by two experts in each field and 42 abstracts were accepted for publication in the proceedings. I wish to congratulate all the authors who are presenting their research work today.

I would like to take this opportunity to thank the YSF steering committee members for their dedication and Dr. Kalpa Samarakoon, Senior Scientists, NASTEC for the generous contribution given in conducting YSF activities.

I hope that the 8th YSF symposium will be a great success.

Dr. Usha Hettiaratchi

Forward by the Editors

It is with great pleasure, the Young Scientist Forum (YSF) present the proceedings of the 8th YSF Symposium. This comprise the research and scholarly activities of the members of YSF.

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 discipline collaborations. It is a place of networking, where constructive scientific feedback is mostly nurtured.

Out of the 50 extended abstracts received for this year, 42 submissions were selected through a double-blind review process after a screening by the editorial board.

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 would is very much thankful to Eng. D.D. Ananda Namal, The Act. Director, NASTEC for funding and facilitating the events of YSF with great enthusiasm. Senior Scientist Dr. Kalpa Samarakoon 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 8th YSF symposium a great success and extend warm wishes to all the authors.

The Editorial Board

POTENTIAL OF SELECTED PLANT EXTRACTS AS HERBICIDE FOR MANAGING THE ALIEN WEED PARTHENIUM (*P. hysterophorus*) IN NORTHERN SRI LANKA

P. Kishojini*, K. Pakeerathan, G. Mikunthan

Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna
**Corresponding author (email: pkishojini@rocketmail.com)*

Introduction

P. hysterophorus is one of the most problematic invasive plant species across the globe. It has been accidentally introduced to Sri Lanka and has since spread aggressively in many regions in Northern Sri Lanka (Jayasuriya, 1999). Therefore, there is an immediate need for controlling this weed from further spreading in this region (Kavinthan 2011). Many weed management techniques implemented were not successful to control this weed fully because of lack of awareness among the farmers and citizens of Sri Lanka as well of its resistance to herbicide, high regeneration capacity, production of huge amount of seeds, high seed germinability and extreme adaptability to a wide range of ecosystems. Research study aimed to identify and select plants-derived products to suppress the growth and spread of *P. hysterophorus* in the northern region of Sri Lanka. The use of botanicals should possibly replace the environmentally harmful synthetic herbicides and is fast becoming important to control noxious weeds. Among the natural plant products, volatile essential oils and their constituents have attracted much attention due to their phytotoxicity (allelopathic properties) and relatively quick degradation in the environment. The volatile substances present in the leaves, released as vapours into the surroundings. Their partial vapour pressure is higher than air pressure; hence, they get adsorbed into soil particles and affect the germinating seeds and seedlings growth.

Methodology

Preparation and application of plants extracts

This study aimed to identify and select plants to suppress the growth and spread of *P. hysterophorus* in northern region of Sri Lanka. Based on phytotoxic properties of plants reported in literatures, twenty potential plants were selected. To get the extraction, fresh, mature and healthy leaves were ground thoroughly and mixed with fermented cow urine for the preparation of extract with different concentrations (25 ppm, 75 ppm, 125 ppm, 175 ppm and 250 ppm) for a preliminary evaluation (Unpublished data). Each extract was mixed with 1 % soap solution Soap solution which acts as a surfactant. The extracts were applied on four weeks old *P. hysterophorus* plants as foliar application using hand sprayer. From the preliminary experiment results, the 175 ppm and 250 ppm doses of *Eucalyptus camaldulensis*, *Allium sativum, Piper betle*, *cassia tora*, *Ricinus communis* and *Tephrosia purpurea* were formulated for the field evaluation. The

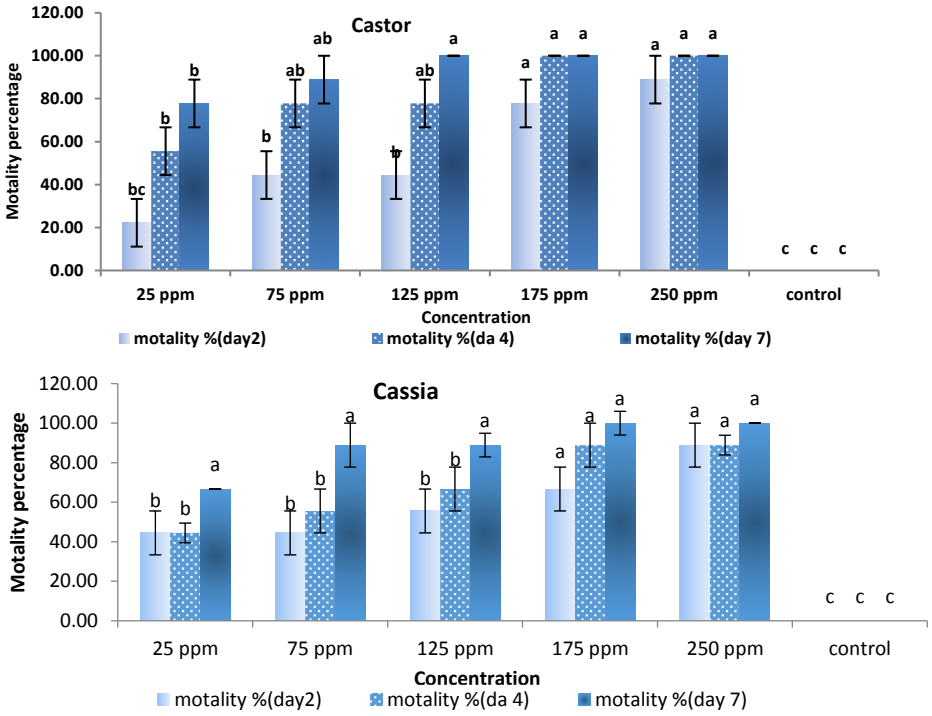
formulated doses were applied on *P. hysterophorus* plants in field using a hand sprayer early in the morning on a clear day. The plants were carefully observed.

Statistical analysis

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

In-Vivo experiment with different botanical extracts showed significant effect on *P. hysterophorus* mortality. The data of *In-Vitro* and field experiments were recorded and mean values are presented in Fig. 1 and 2, respectively. Maximum mortality (100 %) was observed in 175ppm and 250 ppm in Castor followed by Betel and minimum mortality was noted in Garlic (Fig. 1). Castor extract showed highest mortality of 100% at 4th and 7th day after spray (DAS). While 175 ppm *C. tora* extract exhibited 80-100 % mortality which was similar to 250 ppm of the same extract. Betel and *E. camaldulensis* were showed mortality percentage more than 80 % for 175 ppm and 250 ppm concentration in 4th and 7th DAS. But in *T. purpurea* 175 ppm extract showed better effect than 250 ppm. Garlic extract showed comparatively lower lethal effect on this weed than others. Then these two concentrations were selected for the further field experiments.



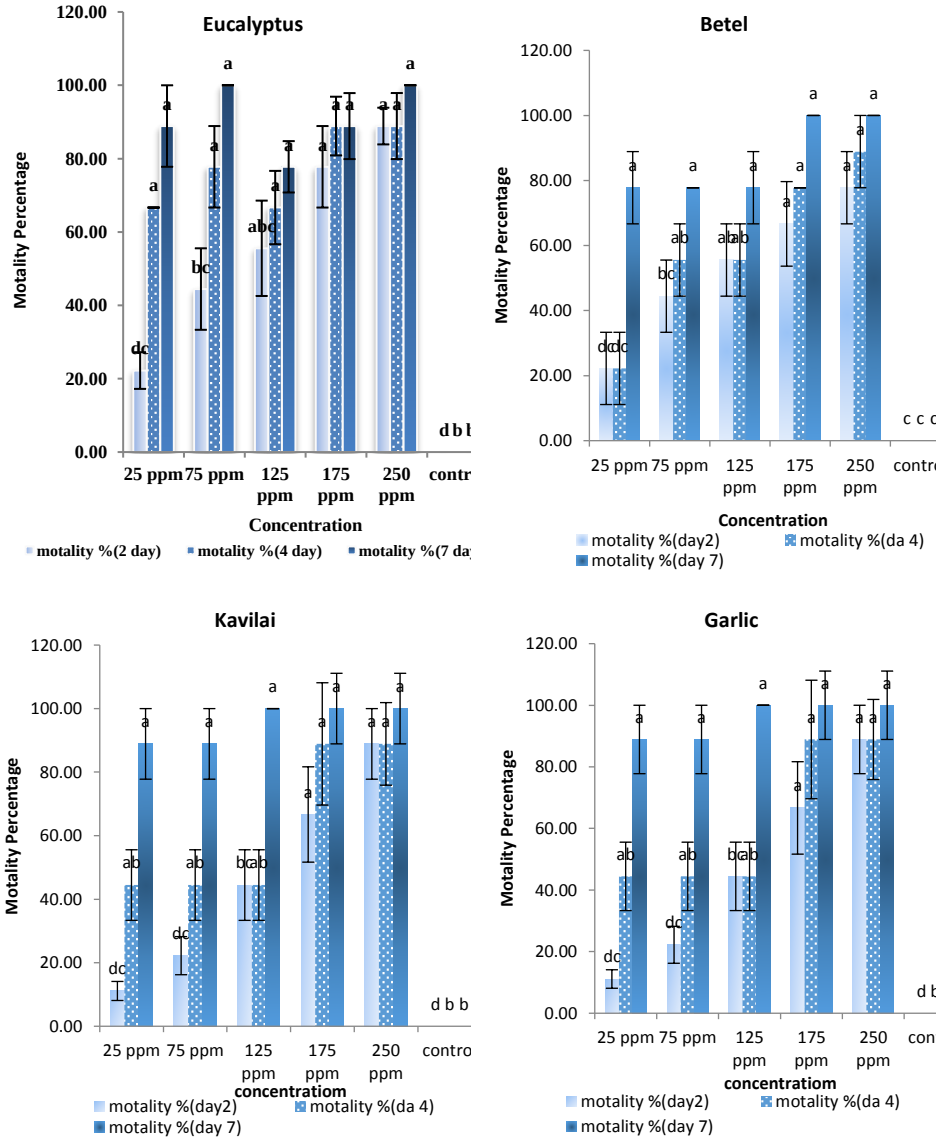


Figure 1. Mortality rate of different plant extract in the pot experiment.

In the field, 175 ppm concentration of Caster and *C. tora* extracts showed better performance to kill the *P. hysterophorus*. But the betel extract lower mortality rate than the other plants. Wilting and necrosis of weed leaves started two days after application. In high concentration (250 ppm), the level of mortality was 90 % to all plants except Betel (Fig. 2). In this concentration too maximum lethal effect was observed in Caster treatment.

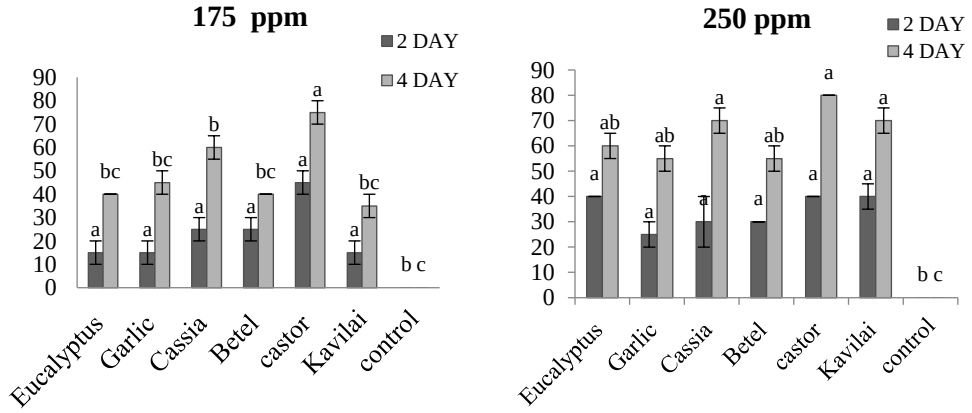


Figure 2. Motility rate of different plant extract in the field experiment.

Many plants excretes chemicals into their growing environments called inhibitors for their successful survival against pathogens. Some of these chemicals even suppress the growth and development of other plants called allelochemicals. *R. communis* showed high mortality rate as compared to rest of the species because it contains allelochemicals which provide suppressive effect. Safdar *et al*., 2013 reported that the aqueous leaf extracts of *R.* communis remained significantly inhibited germination of *P. hysterophorus* weed. Germination and seedling growth inhibition of *R. communis* leaf extract have also been reported by Kong *et al*. (2002) and Jiang *et al*. (2008).

Next lethal percentage was high in *C. tora* in above two concentrations (Fig. 2) at 2nd and 4th DAS. The presence of inhibitory or allelopathic substances in aqueous leachates of *Cassia* affects both the germination and growth of *P. hysterophorus*. The phenolic leachates are responsible for the allelopathic potential of *C. uniflora* for biological control of *P. hysterophorus* weed (Joshi, 1991). Manpreet Kaur (2014) reported that competitive replacement of *P. hysterophorus* can be achieved by planting plants like Cassia species such as *C. sericea*, *C. tora*, *C. auriculata* which suppress the weed growth. Moreover, wholesale propagation of *C. uniflora* was recommended for use in the biological control of *P. hysterophorus* weed (Jai Knox, 2016**).**

Eucalyptus plant extract also showed increased the mortality of this weed in 250 ppm. The volatile terpenes present in leaves of eucalyptus emanate in the form of vapours into the surroundings. The vapours get adsorbed to soil, curbing the seed germination and reducing the chlorophyll content as well as cellular respiration. The oil vapours increase water loss leading to wilting. Eucalyptus oils may be used as natural herbicides for the biocontrol of *P. hysterophorus* owing to its allelochemicals (Kohli et al. 1998).

Conclusion

Selective usage of *R. communis* and C. *tora* extracts as botanical herbicides can manage *P. hysterophorus* and are alternate for inorganic herbicides as well as compatible for integrated *P. hysterophorus* management.

References

[1] Kavinthan S., Thirukkumaran G. and Mikunthan G., Present status of *P. hysterophorus hysterophorus*: Effect of its leaf extract and cow urine on vermicomposting in northern Sri Lanka, B.Sc. Thesis, University of Jaffna, 2011.

[2] Knox, J., 2016. Effect of Cassia occidentalis on *P. hysterophorus* hysterophorus by way of secondary metabolites. *Annals of Plant Sciences*, *5*(11), pp.1451-1453.

[3] Joshi, S., 1991. Interference effects of Cassia uniflora Mill on *P. hysterophorus* hysterophorus L. *Plant and Soil*, *132*(2), pp.213-218.

[4] Kaur, M., Aggarwal, N.K., Kumar, V. and Dhiman, R., 2014. Effects and management of *P. hysterophorus* hysterophorus: A weed of global significance. *International scholarly research notices*, *2014*.

[5] Kong, C., Hu, F. and Xu, X., 2002. Allelopathic potential and chemical constituents of volatiles from Ageratum conyzoides under stress. *Journal of chemical ecology*, *28*(6), pp.1173-1182.

[6] Jiang, H., Gao, X., Zhang, Y., Liu, X. and He, W., 2008. Allelopathic potential of Chinese medicinal plants on rice and paddy weeds. *Allelopathy Journal*, *22*(2), pp.337-343.

[7] Kohli, R.K., Batish, D.R., Singh, H.P. and Dogra, K.S., 2006. Status, invasiveness and environmental threats of three tropical American invasive weeds (*Parthenium hysterophorus* L., *Ageratum conyzoides* L., *Lantana camara* L.) in India. *Biological Invasions*, *8*(7), pp.1501-1510.

[8] Jayasuriya, A.H.M. 1999. Weed alert. *Parthenium hysterophorus* in Sri Lanka. Plant Protection Newsletter 3(2). Postgraduate Institute of Agriculture, University of Peradeniya. ISSN 139 1- 345X.

EFFECT OF SEMI-AUTOMATED DEFICIT IRRIGATION ON YIELD PARAMETERS OF EGGPLANT (*Solanum melongena L.)*

S. Sayanthan* and N. Kannan

Department of Agricultural Engineering, University of Jaffna, Jaffna, Sri Lanka
**Corresponding Author (email: mylvaganam90@gmail.com)*

Introduction

Water is a vital and scare resource. It is reported that within the available water around 90% is being withdrawn for irrigation purposes. Nowadays, as a result of over exploitation the relative amount of water available for the agriculture sector, abating in a faster rate. Therefore, it is important to adopt some rational approaches in water application, to sort out the issues related to excess water diminution.

Different tactics of irrigation scheduling involve measuring of soil, plant and environmental factors to determine when and how much water to apply. However within all parameters soil related measurements; soil moisture sensing is more popular which uses dielectric properties of the soil as it is auto-dynamic; not affected by the environmental manipulations and easy accessible (5)

There are many more evidence that the application of water less than the plant requirement will pushes the plant in to stress and reduce the growth and yield of plants. Kirnack et al. (2002) demonstrated that the deficit irrigation in eggplant leads to reduced amount of average weight of fruit and lowered plant height and diameter (2).

Even though the deficit and excess irrigations have their own pros and cons, our objective was to compare the pre-determined levels of deficit and over irrigation conditions in terms of yield parameters such as total yield, fruit length, fruit diameter and single fruit weight.

Materials and methodology

Establishment of device

In order to establish a device to measure soil moisture, a gadget was prepared in arduino platform with proper modules sensors and code.

Plant selection

For the research work, eggplants were selected, due to the leading contribution in Jaffna districts' vegetable production for last consecutive years. Selected eggplant was a selection called "plastic"

Field preparation and Preliminary investigation

A field was selected in Department of Agriculture farm, Thirunelvely. According to the Agriculture department spacing recommendation, drip system and basin

irrigation system were developed in the field and some preliminary investigations such as infiltration rate, field capacity level of the soil, emitter discharge, emission uniformity coefficient and finally the measurement of quantity of water being irrigated by the farmers in different stages of eggplant were carried out.

Field planting

Eggplants were planted in three adjacent partitions. Third plot was allowed to have basin irrigation and other two were with drip irrigation. Each field were allowed to have 6 columns and each Colum consist 13 planting points. Planting was carried out in two different times. First field was planted 31 days prior to the planting of second and third field. First field was used for the root investigation and the second field was the test field and the third field was the control field.

Root investigation

The first field was irrigated according to the recommendation of Department of Agriculture training center. From each column of root investigation field, 4 plants were randomly selected and uprooted according to the monolith method and roots were investigated for vertical and horizontal lengths.

Irrigation

The test was planned as reduced level of water application up to 70% of the total water requirement, in order to achieve that level of irrigation, the top half part of the plant root zone was maintained at field capacity level with the help of prepared moisture sensing gadget via the drip system and it was re-irrigated from 50% depletion level. The control field was planned to irrigate with the quantity and irrigation interval of water application same as the farmers' practice

Measurements and analysis

Total yield from test and control fields were measured. More over average length and weight of a single fruit also measured. Measured parameters were statistically analyzed via the independent t-test.

Results and discussion

Obtained Discharge rate was 3.6 l/h at the pressure of 1.2 kg/ cm^2. Calculated emission uniformity coefficient was 88.88%, this records ensures the acceptability of used drip system in the field as they are highly uniform and makes sure that all planting hall will receive equal quantity of water

Measured infiltration rate of the soil was 350mm/h therefore, our soil lies under very rapid infiltration soils and chances for flooding during irrigation via drip will be nil.

Finally the estimated field capacity by using soil moisture gadget, of the soil was 28% and the permanent wilting point was taken as 10%

The measured amount of water being applied for a single plant up to 108 days after planting, by the farmers was around 604.8 liters. The quantified water, needed by a single plant in order to compensate the 70% of plant water requirement up to 108 days after planting was around 104.64 liters.

According to the chart 1, deficit irrigation conditions significantly affected the plant total yield and according to chart 2, 3 and 4, Average fruit weight, fruit length and fruit diameter were significantly reduced in test field under deficit irrigation as a result of this effect marketable yield was reduced in deficit irrigation regimes. Same result was obtained in the research carried out by Mitchel *et al.* (1991) in tomato (3) and Smittle *et al*. (1994) in bell pepper (4). The better performance by the crop from the control field was due to result of the better maintenance of internal water balance by the plants and improved utilization of water and dissolved nutrition.

The fruit size and weight reductions are the visible result due to insufficient soil moisture in plants' root zone. Whenever the soil moisture tension increases due to law water availability, there will be a reduction in the plant yield parameters. The ideal result was obtained by Smittle *et al.* (1994) in bell pepper along with the reductions in growth and turgidity (4). When we go for the production per liter, the test field was 8.4 times better than the control field, because, the increased efficiency of water use under stress is because that drought-stressed plants wilt far more than unstressed plants and wilting invariably occurs in times when the saturation deficit of the atmosphere is large. Therefore, the plant assimilates only in times when the saturation deficit is small and hence loses low water quantity for every carbon molecule fixed (1).

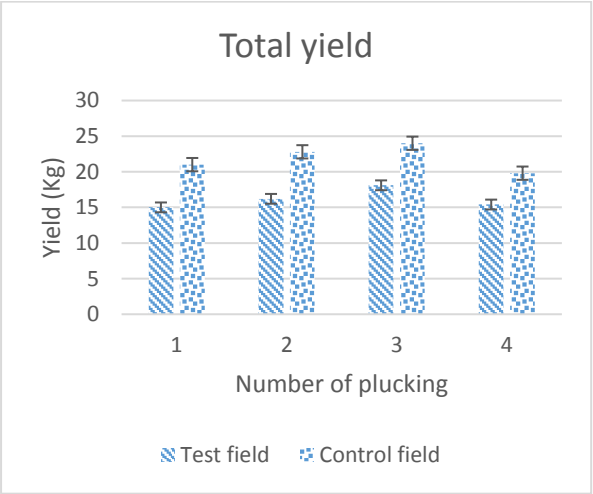


Figure 1: Total yield during different plucking in test and control field

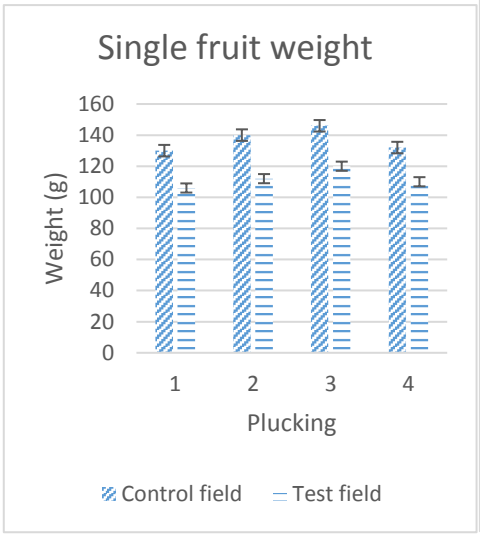


Figure 2. Single fruit length during Different Plucking in test and control field

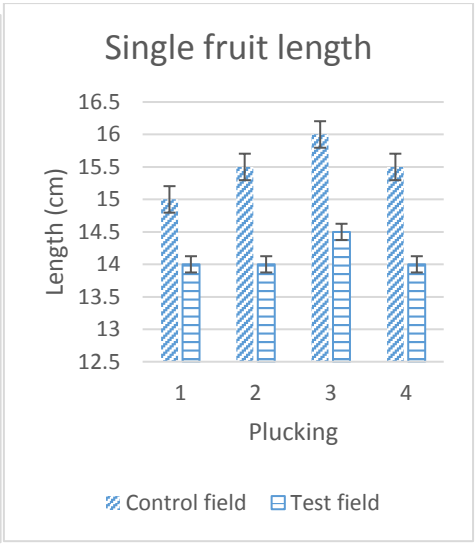


Figure 3. Single fruit weight during different plucking in test and control field

Conclusion

Results obtained in this research study clearly explains successful deficit irrigations strategies can be carried out with the support of process control techniques such as using soil moisture sensors. The significant difference in all yield parameters between the test and the control portrays that the drought – stress conditions always exert a negative impact on the yield parameters of eggplant. But the productivity, in terms of yield per liter is 8.4 times higher for deficit irrigation applied up to 70% of total water requirement compare to the over irrigation condition. Therefore with the focus of maximizing the yield with the concern of water sustainability, the deficit irrigation regime will be the better practice.

Acknowledgement

Authors thank the Research Grant 2016 of the University of Jaffna for the financial support and Mr.N.Thaneswaran, senior staff technical officer, for his immense support.

References

[1] Clover, G.R.G., Jaggard, K.W., Smith, H.G., Azam-Ali, S.N., 2001. The use of radiation interception and transpiration to predict the yield of healthy, droughted and virus-infected sugar beet. J. Agric. Sci. Camb. 136, 169–178

[2] Kirnak, H., Tas, I., Kaya, C., Higgs, D., 2002. Effects of deficit irrigationon growth, yield and fruit quality of eggplant under semi-arid conditions. Aust. J. Agric. Res.53, 1367-1373.
[3] Mitchell, J.P., Shennan, C., Grattan, S.R., 1991. Developmental changes in tomato fruit composition in response to water deficit and salinity. Physiol. Plant. 83, 177-185.
[4] Smittle, D.A., Dickens, W.L., Stansell, J.R., 1994. Irrigation regimes affect yield and water use by bell pepper. J. Am. Soc. Hortic. Sci. 119, 936-939.
[5] Tüzel, I.H., Tüzel, Y., Öztekin, G.B., Meric, M.K., Whalley, R. and Lock, G. 2009. Response of cucumber to deficit irrigation. Acta Hortic. 807, 259-264.

INCIDENCE OF MAMMALIAN PEST ATTACKS ON RUBBER CULTIVATION

M.K.R. Silva*, T.H.P.S. Fernando, B.I. Tennakoon

Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta, Sri Lanka
Corresponding Author (email:** *keshanisilva@yahoo.com)**

Introduction

Incidences of mammalian pest attacks which were of little concern few decades ago have become a routine complaint of the growers of both estates and smallholder sector nowadays. As a result of the invasion of the wild habitats by rubber and other crops such as tea, oil palm and cinnamon, wild mammals have started invading these cultivated lands for their food and other needs. This is evidenced by most of the reported mammalian pest damages being from areas bordering shrub jungles. Due to the attacks made by mammals, young rubber plants may be totally destroyed while mature trees also could be led to death due to the ring bark condition.

In order to streamline the management strategies, it is important to identify the nature of the problem of mammalian pests and the significance imposed by this on the rubber cultivation. The objectives of the current study was to identify the different mammalian species which cause damages on rubber, the type of the damage caused by each species and to recognize the significance of the damage on each rubber cultivation. Further, the evaluation of the adaptability and the effectiveness of the conventional management strategies was also an objective.

Materials and methods

Data collection

The set of reported mammalian pest attack incidents by the Department of Plant Pathology and Microbiology, Rubber Research Institute of Sri Lanka for a period of two years was used as the sample of the study. Observations and unstructured questionnaire were used to gather the information on each incidence. The mammalian species and the type of damage caused by each species, was identified using the observations made and with the information provided by the growers.

The number of damaged rubber trees upon the total number of rubber trees in each specific clearing was considered as the significance of the damage caused on the particular rubber clearing. The different management strategies currently adopted in the field were identified and the adaptability and the effectiveness of them were evaluated through observations as well as based on the information provided by the growers.

Statistical analysis

Simple statistical methods and descriptive tools: viz means, tables, and figures were used to analyze the data.

Results and Discussion

1. *Type of the mammalian species and the nature of damage caused by each species*

Most of the complaints had been on damages due to porcupine and wild boar. There were incidences with damages due to both creatures (Figure 01). Out of the 37 incidences, 62.5% was solely due to porcupine and 18.75% was due to wild boar. In 15.63 of the cases, mixed invasions were observed. The age of the rubber plantation having damage was between six months and six years. No damage on mammalian pest attack was observed from trees under-tapping. The nature of the damages caused by these species was remarkably different.

Porcupine

The porcupine attack has been reported from the time of establishment up to tapping of the rubber tree. In the very early stages of the rubber plant, it had uprooted the plant and fed on the immature underground parts and consequently a high percentages of casualties had been resulted (Figure 01 a). At later stages, they feed on the bark of the rubber trees and the damage exhibit the characteristic biting marks on the remaining wood surface (Figure 01 b, c). It is easily distinguishable from the damages of other species due to these marks. In some cases, the tree had been totally ring barked leading to the death (Figure 01 d).

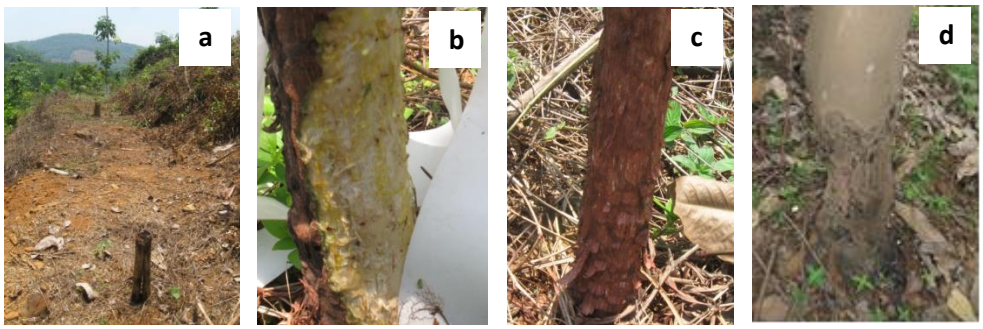


Figure 01. Porcupine attack on rubber

(a) vacancies caused by porcupine attack in an immature rubber field
(b) a freshly damaged bark: note the characteristic biting marks on the wood
(c) an old damaged bark: note the characteristic biting marks on the wood
(d) ring-barked condition in a mature tree

Porcupines are herbivore and a fossorial rodent species and a serious pest of forest plantations and agricultural crops in many countries of the world (Alkon and Saltz, 1985; Sheiker, 1998; Idris and Rana, 2001). Porcupines eat almost any plant material: bark, branches, leaves, buds and twigs and tendency of eating parts of the rubber tree can be attributed to their salt-loving nature and poisoned salt blocks were evaluated for effectiveness in killing porcupines (Anthony *et al,* 1986).

All the complaints on the porcupine attack were received from rocky rubber lands, where poor weeding was practiced. In majority of cases, it was observed that, the burrow systems had been developed in association with the small-to-medium rocks present within the particular rubber land and in such cases; the damage was present since the establishment of rubber. The porcupines are strictly nocturnal animals that live in extensive burrow system, scattered in the dinning habitat, and are occupied by one or more family groups. They regularly come out of the burrow to forage, usually during night, as forage is not stored in the burrows (Roberts, 1997; Mian *et al*., 2007).

In some cases, sudden outbreaks, 2-3 years after the establishment have been reported due to recent clearance of an adjacent land from where the creatures invade the rubber land.

Wild boar

Wild boar attacks on the rubber tree had been observed from the time of establishment up to tapping. Wild boars had not ingested any plant/ tree part. However, they have caused a variety of damages; most common was rooting (grubbing) of the very young plants causing total destruction. Severe damages had been caused to the bark of the adult trees by punching with tusks due to their aggressive behavior. Those damages resembled the cutting marks made by sharp equipment such as a knife (Figure 02). Consequences of the damage had sometimes ended up with the ring barked situation causing the death of the tree. Moreover, after-effects such as growth retardation, increasing proneness to wind damage and invasion of secondary microorganisms had occurred even after the recovery of the lesion.

Figure 02. Adult trees damaged by wild boar: note the punching marks and the mud applied on the bark

It has been observed that the damage incidences are more frequent after a spell of rain in the area. However, it is difficult to use this as a rule of thumb in adopting management strategies, because, other than the major spells of rain, light rains experienced in localized pockets may also aggravate the attack.

Wild boar is one of the most widely distributed mammals in the world and they have the highest reproductive rates among ungulates, and their local density can double in one year (Erkinaro *et al*, 1982).

2. The Significance of the damage due to mammalian pest attacks

As this study does not cover the whole set of mammalian pest incidences in the country throughout the period, no evaluation could be done on the national significance of the problem. The number of incidences would be much higher than the 37 number of reported incidences for a period of two years. Therefore, the incidental significance was assessed in each case. When a particular rubber clearing is considered, the damage percentage ranges from 5% to 100% of the total number of plants/ trees present in the clearing. 86% of the incidences were on rubber clearings of smallholder sector and the rest were on the rubber lands under the Regional Plantation Companies.

Apart from the direct significance of the damages, there were indirect effects of the mammalian pest damages. Even after the wound recovery, growth retardation of the attacked trees was observed. The invasion of secondary microorganisms from the point of recovered lesion was also noticed (Figure 03 a). The development of off shoots in damaged trees was also had resulted (Figure 03 b). Moreover, the conventional practice of applying mixtures of cow dung, sulfur and clay on the wound may create avenues for the infection at the wound (Figure 03 c). Furthermore, the malpractice of applying Candarsan on the bark with the aim of repelling mammalian pests had caused the scorching of the bark and the sudden death of the several young rubber trees in one incidence. As collective result of all these negative impacts, the reduced productivity attributed by the damage due to mammalian pest has led to ruined smallholders' perception on rubber cultivation.

Figure 03. Indirect effects of mammalian pest attacks
(a) development of secondary microorganisms
(b) development of off-shoots

(c) infections occurred due to the applying of cow dung, clay and sulfur on the wound

3. Conventional Management strategies

A variety of conventional methods are being used for protecting plants from the mammalian pests, out of which some are effective to a certain extent. Most of the other conventional management strategies practiced in the field were based on the local availability of different materials which are used as excluders. Establishment of bamboo strips, plastic drink bottles, gunny bags, poly sacks, old buckets, old metallic roofing sheets and roofing tiles and encircling the plants with white polythene bands or stripes, were some of the conventionally used practices. Covering the animal paths with fish nets and spreading cut hair pieces around the rubber trees were also used as excluding techniques. Hunting of the creatures, were also practiced in certain locations.

It was observed that, some of these methods were effective based on the success of covering the rubber plant. As the durability of such material is low, repeated application was needed, so that the cost for labour has to be taken into account. However, in the small holdings where home labour is basically used this makes no issue. When the material cost is also not exerted, (as locally available materials are used) the use of these conventional strategies is cost effective. However, it was observed that some of these measures have their own adverse features and therefore special attention was required when employing such measures. Bamboo pieces aggravate the termite problem and are not durable. Heating of the metallic roofing sheets could cause adverse effects on the growing rubber plant and therefore care should be taken to leave enough space between the plant and the sheet. When using these excluding materials, awareness on the type of the attacking creature, its habitat and the point of damage to the rubber plant is required.

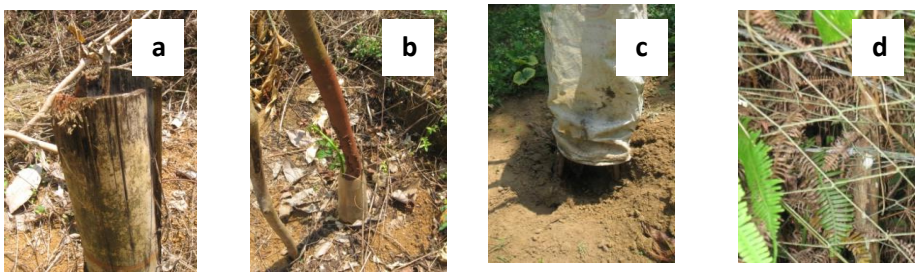


Figure 04. Failures occurred due to inappropriate application of conventional methods

(a) covering plants with bamboo
(b) covering plants with plastic bottles
(c) covering plants with poly sacks
(d) covering the fence with old fish nets

Failures of conventional strategies were frequently observed due to such problems (Figure 04). When the attacking creature is porcupine, fencing is not effective. Moreover, the height bamboo strips and plastic drink bottles is critical (Figures 05a, 05b). When a poly sacks are used as excluders for porcupines, attack has been made on the immature underground parts (Figure 05c). It is not effective to cover the fence with old fish nets, when the land is rocky and the creatures are living within the land (Figure 05d).

Conclusions and recommendations

Out of the results of the study, it can be concluded that the menace of mammalian pests has become a significant problem in the industry throughout the period of consideration. Porcupine and wild boar are the most common types of mammalian pest which may cause even the death of the trees on rubber. Various conventional efforts have been adapted to manage the problem. Site-specific management strategies have to be used in order to carry out an economic and successful management programme. Moreover, it should be noted that an effective management of mammalian pests can only be achieved by integrated action over a large area otherwise animals will continually reinvade from the neighboring areas.

References

[1] Alkon, P. U., Saltz, D., 1985. Patterns of crested porcupine (*Hystrix indica*) damage to cultivated potatoes. Agric. Ecosystem Environment, 14: 171-183.

[2] Anthony, R.M., Evans, J., Lindsey, G.D. 1986. Strychine –salt blocks for controlling porcupines in pine forests: Efficacy and Hazards, Proceedings of the Twelfth Vertebrate Pest Conference.

[3] Erkinaro, E., Heikura, K., Lindgren, E., Pulliainen, E., Sulkava, S. 1982. Occurrence and spread of the wild boar (*Sus scrofa*) in eastern Fennoscandia. 58: 39-47

[4] Roberts, T.J., 1997. The mammals of Pakistan (revised ed.). Oxford University Press, Karachi, Pakistan: 525.

INFLUENCE OF DIFFERENT POTTING MEDIA ON GROWTH OF *Codiaeum variegatum*

B.G.S.M. Wijebandara and R.D.N. Debarawatta*

Department of Horticulture and Landscape Gardening, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), 60170.

**Corresponding author (email: dinushand@wyb.ac.lk)*

Introduction

Floriculture has become a profitable sub-sector of agribusiness throughout the world in recent years. The climatic variation coupled with a diverse terrain enables Sri Lanka to develop a wide range of floriculture flora. The export trade in floriculture began in early 1980s, by now it has grown substantially to become one of Sri Lanka's major foreign exchange generating ventures.

Croton (*Codiaeum variegatum*) are varied plants that are often grown as houseplants. It is native to Malaccan Islands, between the Philippines and New Guinea. *Codiaeum variegatum* is the second largest genera of the family Euphorbiaceae. There are only six known basic species of *Codiaeum* of which, all other cultivars arose as mutants or hybrids. *Codiaeum variegatum* is one of the most popular ornamental plant and it is an evergreen shrub, up to 6 m in height. In addition to its aesthetic value it is used as an interior plant. Foliage plants are often used as indoor plants because of their attractive foliage and their ability to survive and grow under limited indoor light [1].

There is a continuous interest of using different agricultural by-products as organic nutrient sources for plants due to increasing awareness of environmental related issues, as well as the need to dispose the rising waste. Recycling organic wastes including dung of dairy cattle, poultry waste and animal litter are the main sources of organic wastes. Those organic waste and compost can be used for the cultivation of *C. variegatum* instead of chemical fertilizers [2].

Codiaeum variegatum plants have attained a prominent place among foliage plants because of their adaptation to indoor conditions. Ornamental value of *C. variegatum* is completely depending on potting media, because this media play a vital role in the growth and production. Light, nutrient rich, well-drained soil with pH slightly below or above the neutral point is considered as the ideal media for *C. variegatum* cultivation[2].

The Department of Agriculture has recommended a common potting media for foliage plants cultivation and there is no any specific potting media was found in Sri Lanka for *C. variegatum* cultivation. Further, nursery people mentioned that laterite soil mixed potting mixture was favourable for *C. varigatum* cultivation. Therefore, present study was conducted with the aim of investigating the suitable potting mixture for the *C. variegatum* cultivation.

Materials and Methods

Experimental Site

The study was conducted at Faculty of Agriculture and Plantation Management Wayamba University of Sri Lanka, from May to January 2018.

Planting Material Collection

Healthy and uniformly grown plants of *C. variegatum* were collected from the University premises and Makandura area and Semi hardwood cuttings were obtained for the study. Length of a cutting was 15 cm with ±25 buds/cutting.

Field Experiment

Semi hardwood cuttings were planted in black color polythene bags (19 cm × 10 cm, gauge = 300) in four different potting mixtures. Potting mixtures were pre moistened before planting the cuttings and base of the cuttings were treated with 3-Butric Acid (0.3% I.B.A). Two hundred cuttings were used for the experiment (Table 1). Each potting mixture represented 50 cuttings. Treatments were arranged according to the Complete Randomized Design (CRD). Poly bags were placed inside a propagator covered with transparent polythene (gauge 500, relative humidity 73% and temperature 30 ^{0}C). Watering, weeding and other agronomic practices were practised regularly.

Table 1. Composition of potting mixtures, bulk density and moisture availability

Treatment	Potting mixtures	Moisture percentage	Bulk density
T 1	TS 4 : C 2 : DCM 1 : S 1	31.64	1.2695
T 2	TS 2 : C 4 : CD 2 : LS 1	41.15	1.2425
T 3	TS 2: C 2 : LS 1	30.43	1.2801
T 4	TS 2: C 4 : CD 1 : S 1	32.60	1.2801

(Note : TS- Top Soil, C – Compost, DCM – Decayed Cattle Manure, S – Sand, CD – Coir Dust, LS – Laterite Soil)

Data Recording and Analysis

Moisture percentage and bulk density was recorded in four different potting mixtures. Two months and five months after planting (MAP), total plant height, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight (oven dried 80^{0}C for 48 hours) were recorded. Data were analysed using one way ANOVA and mean separation was done by Tukey's test, using Minitab 16 software.

Results and Discussion

Mean Plant Height

There was no any significant difference of plant height of *C. variegatum* plants, at 2 MAP ($p<0.05$). Further, at 5 MAP there was no any significant difference of plant

height in T 2 and T 4 potting media. But, there was a significantly difference of plant height was recorded in T 1 and T 3. It resulted that, the vegetative performance of *C. variegatum* plants were increased in 5 MAP stage compared with the 2 MAP stage in different potting mixtures. (Figure 1).

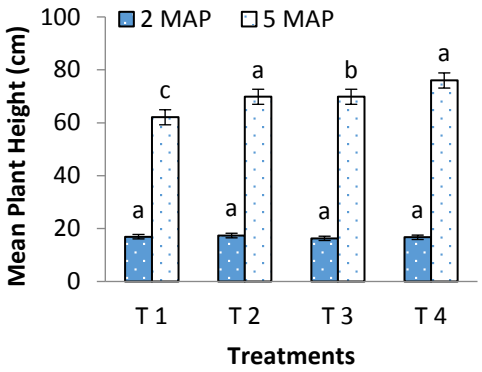


Figure 1. Mean plant height of *C. variegatum* grown in different potting mixtures *(MAP – Months After Planting)*

Mean Shoot Fresh Weight

When concern about the shoot fresh weight, there was no any significant difference ($p<0.05$) among the potting mixtures at 2 MAP. Further, at 5 MAP, there was a significant different of mean shoot fresh weight of T 1 when compared with other treatments. Furthermore, among T 2, T 3 and T4 potting mixtures the highest mean fresh weights of shoots were recorded in T 2 (86.23g) (Figure 2).

Mean Shoot Dry Weight

According to the Figure 2, there was no any significant difference ($p<0.05$) of mean shoot dry weight among the treatments at 2 MAP and 5 MAP. While, the highest value was recorded in T 2 at both 2 MAP and 5 MAP (Figure 2).

Mean Root Fresh Weight

In *C. variegatum*, there was a significant difference ($p<0.05$) of mean root fresh weight at 2 MAP in T 2 potting media when compared with other potting mixtures. Further, there was no any significant difference among T 1, T 3 and T 4.However, at 5 MAP, there was a significant difference of root fresh weight when compared with T 1 while, there was no any significant difference of root fresh weight among T 2, T 3 and T 4.But the highest mean root fresh weight (13.4g) was recorded in T 2 potting media (Figure 3).

Mean Root Dry Weight

There was no any significant difference ($p<0.05$) of mean root dry at 2 MAP of *C. variegatum* plants (Figure 3). Further, the highest mean root dry weight value was revealed in T 2 (0.3702 g). Similarly, there was no any significant difference of

root dry weight of *C. variegatum* plants at 5 MAP. Furthermore, the highest value was recorded in T 2 (3.02 g) when compared with other treatments (Figure 3).

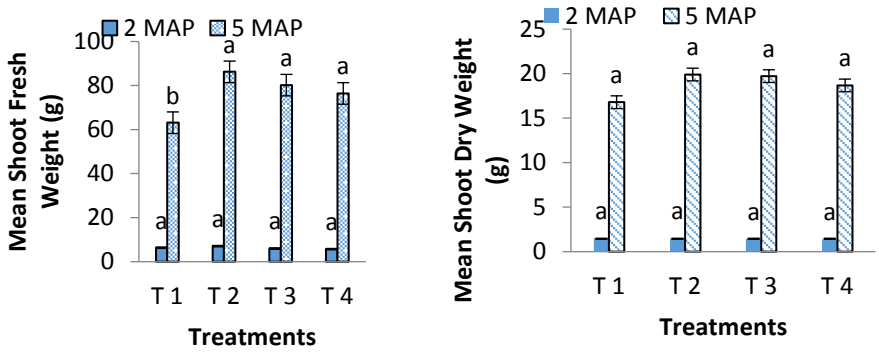


Figure 2. Mean shoot fresh weight and dry weight of *C. variegatum* grown in different potting mixtures *(MAP – Months After Planting)*

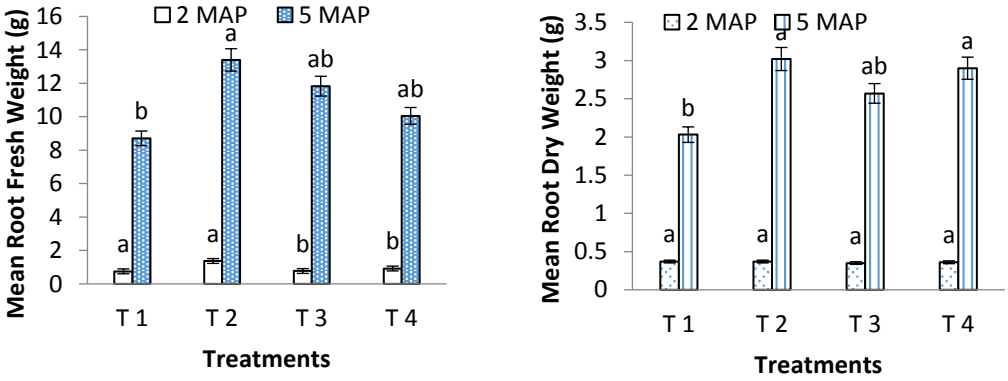


Figure 3. Mean root fresh weight and mean root dry weight of different treatments

Potting medium is an important factor that plays a key role in the production of quality *C. variegatum* plants production. Department of Agriculture has recommended a common potting mixture for foliage plants. However, there is no any specific potting media for the growth and development of *C. variegatum*. Further, similar study reported that sand: silt: leaf compost: spent compost of button mushroom production with 1:1:1:1 ratio mixture is better for the *C. variegatum* cultivation [2]. According to the Sri Lankan conditions, leaf compost and spent compost of button mushroom media are difficult to find in large scale cultivation. Therefore, for the commercial scale cultivation, it is important to investigate a suitable potting mixture for the *C. variegatum* cultivation which was readily available in the Sri Lankan condition.

According to the present study at 5 MAP, the highest value mean shoot fresh weight and mean shoot dry weight was recorded in T 2. Further, significantly the highest mean root dry weight and fresh weight was also recorded in T 2. Furthermore, the highest moisture percentage also recorded in T 2. The quality production of ornamental plants can be attained by the use of appropriate potting media, which have a prominent effect on growth [3] further, the growing media directly affected for the root growth and development of plants.All potting mixtures have contributed for the vegetative growth and development of *C. variegatum* plants. Present study resulted that the highest growth performances was recorded in T 2 at both 2 MAP and 5 MAP stages. Therefore T 2 (Top soil : Compost: Coir dust : Laterite soil with 2 : 4: 2 : 1) can be suggested as the best potting mixture for the *C. variegatum* cultivation.

Conclusion and Recommendation

Codiaeum variegatum is the most popular plant in the family Euphorbiaceae. It has a high demand in the local market and export market. *Codiaeum variegatum* is widely exported as rooted cuttings and cut leaves from Sri Lanka. In order to gets an economical and high-quality production of planting materials for the exportation, potting media play a major role. Hence, the present study revealed that, the top soil: compost: coir dust: laterite soil with 2:4:2:1 ratio potting mixture was able to generate the highest growth performances than other potting mixtures. Therefore, top soil: compost: coir dust: laterite soil with 2:4:2:1 ratio can be recommended as the best potting mixture for growth and development of *C*. *variegatum*. However, studies should be continued to evaluate the variation of growth performance of potting mixtures with time.

References

[1] J. Chen and R.J. Henny. “Recent advances in plant biotechnology and its applications, Ornamental foliage plants: Improvement through biotechnology”, I.K. International Publishing House, New Delhi, India, 2008, pp. 140–156.

[2] A. Younis, R. Atif, M. Waseem, M.A. Khan, and M. Nadeem,“Production of quality croton (*Codiaeum variegatum*) different plants by using growing media”, *American-Eurasian Journal of Agriculture and Environmental Science*,vol.7 (2), pp. 232-237, 2010.

[3] A.W. Vendrame, I. Maguire. and K.K. Moore. “Growth of selected bedding plants as affected by different compost percentages”. Proceedings of the Florida State Horticultural Society,2005,p.118: 368- 371.

EFFECT OF VOLATILE METABOLITES PRODUCTION BY ANTAGONISTIC FUNGI, AGAINST *Rigidoporus microporus*, THE CAUSATIVE PATHOGEN OF WHITE ROOT DISEASE IN RUBBER LANDS

P.W. Balasooriya[1, 2], T.H.P.S. Fernando[1]*, O.V.D.S.J. Weerasena[2]

[1]*Department of Plant Pathology and Microbiology, Rubber Research Institute of Agalawatta, Sri Lanka*
[2]*Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka*
**Corresponding author (email:thpsfernando@yahoo.com)*

Introduction

Natural Rubber is playing a significant role in Sri Lankan economy. To maintain sustainable rubber cultivation it is necessary to control disease. White Root Disease (WRD) is the most destructive root disease caused by *Rigidoporus microporus*, in the rubber plantations. In Sri Lanka it has been estimated that 5 – 10% of the cultivated rubber lands are under bare patches due to this disease and area affected is increasing at an alarming rate [1]. Several fungicides have been tested against the white root disease. However, the fungicides recommended for treatment are expensive and continuous use of chemicals can be toxic to the environment. Recently, biological control is becoming an increasingly potential alternative control measure to replace chemical fungicides [2],[3]. Among the mechanisms use to control the rival by the antagonistic fungi, the toxic secondary metabolites production is more prominent. They can produce various kinds of diffusible and volatile compounds with strong inhibitory activity against plant pathogens. Moreover, volatile compounds can promote plant growth with antifungal activity [3]. As the fungal volatile compounds are naturally occur, the harm to environment is much lower than artificial fungicides. Due to the diffusible ability of volatile metabolites, pathogen growth can inhibit without direct contact with antagonist. The main objective of this experiment is to identify volatile secondary metabolites production ability of antagonistic fungi against *R. microporus*.

Methodology

Isolation of soil fungi

In order to find the antagonistic fungi from rubber lands in Sri Lanka, soil samples were collected from Kalutara, Rathnapura, Monaragala, Ampara, Polonnaruwa and Vavuniya districts using random sampling method. Using dilution plate technique soil fungi were isolated and visually different fungus were used to further studies.

Selection of best antagonistic fungi

Pathogen center test was used primarily to identify the antagonistic ability of the isolated fungi. *R. microporus* disc (5mm) was placed in the center of the PDA plate. Four discs of each fungi (5mm) were placed with the distance 3.5mm away from the pathogen disc to form a square. All the plates were incubated at 28 ± 2ºC under natural light and dark condition

The effective fungi were subjected to dual plate culture method and confirmed the antagonistic ability against *R. microporus* [4]. Best antagonistic fungi were isolated according to the results obtained from the dual plate culture test.

Calculation:

$$I = \frac{R1 - R2}{R1} \times 100\%$$

Where, I =Percentage of inhibition
R1 =Radius of the pathogen in control
R2 =Radius of the pathogen towards the antagonist

Volatile metabolite production by effective antagonistic fungi

To evaluate the capability of the fungi to control the pathogen using volatile metabolites, mycelia disc (5mm) of antagonistic fungi was placed on the center of the PDA plate and incubated for 4days. After 4days a disc (5mm) of *R. microporus* was placed on the center of another PDA plate. Then the lid offed pathogen and antagonistic fungi containing plates were placed with each other's bottom and sealed to avoid gas exchange with surround. The radial growth of pathogen was recorded after 5 days. Control was arranged without antagonistic fungi. The percentage inhibition related to the control was calculated using above equation [5].

Results and Discussion

Basically more than eighty different fungal species were isolated from soil samples. After subjecting those to pathogen center test forty-five isolates were selected as effective antagonistic fungi. According to the dual plate culture test twenty-three best antagonistic fungi were selected which showed more than 60% inhibition (figure 1). These selected fungi may be an environmental friendly solution to control the White Root Disease.

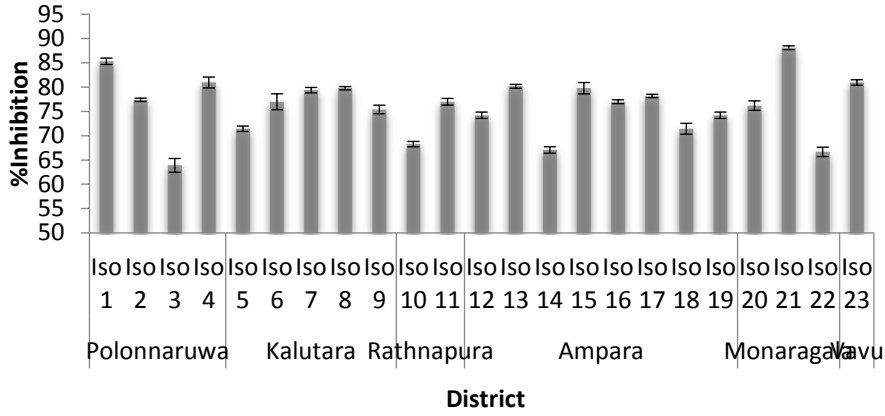


Figure 1. Percentage inhibition of antagonistic fungi against *R. microporus*

According to the colony morphological and microscopical observations, isolate 01,03 & 21 were identified as *Aspergillus* spp, isolate 02, 08, 09, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20 and 23 as *Trichoderma* spp and the rest were unidentified.

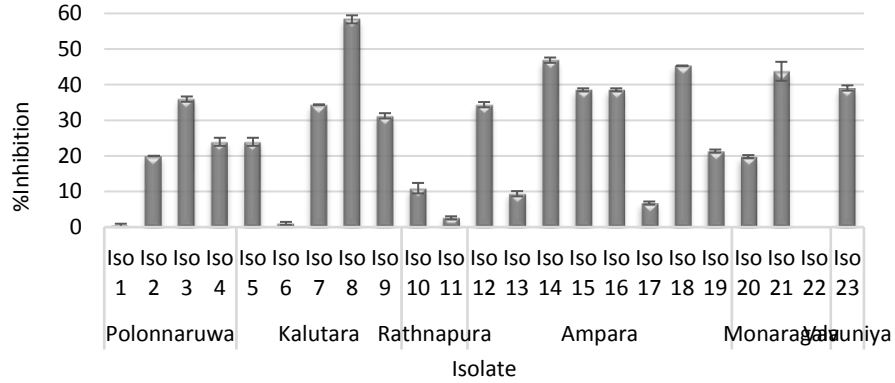


Figure 2. Percentage inhibition due to Volatile metabolite production of antagonistic fungi

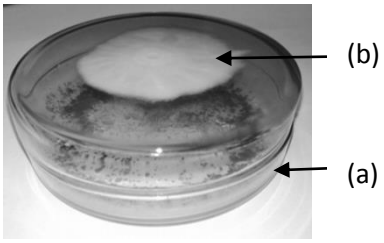


Plate 01: The volatile effect of antagonistic fungi. Antagonistic fungi at lower plate (a) and pathogen at upper plate (b).

The highest percentage inhibition due to volatile metabolites was observed in Isolate 8, which was isolated from Kalutara district rubber land. Except isolate 22 all other isolates were contributed to show an inhibition of pathogen by producing volatile metabolites. Isolate 08, 14, 18 and 21 showed more than 40% inhibition of *R. microporus*. Although other isolates were given less than 40% inhibition, there was a significant impact to control the pathogen. Except the Isolate 01, other two *Aspergillus* spp (Iso 3 and 21) were recorded significant inhibition against the pathogen.

Conclusion and recommendations

Volatile metabolite production is an extra benefit to control the *R. microporus* by the antagonistic fungi. As well as the diffusible metabolites and fungal cell wall degrading enzymes, volatile metabolites also contribute to control the pathogen. By using combination of these microorganisms can be improved the effectiveness of the overall result.

References

[1] W.P.K. Silva. "White root disease caused by *Rigidoporus microporus* in Sri Lankan rubber plantations", Proceedings of International Workshop on White Root Disease of *Hevea* Rubber, Colombo, Sri Lanka, December, 2010, p.28-31.

[2] L.S. Thomashow and D.M. Weller. "Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites". *Plant-microbe interaction Springer US*, pp187-235, 1996.

[3] Arrebola, Eva, Dharini Sivakumar, and Lise Korsten. "Effect of volatile compounds produced by Bacillus strains on postharvest decay in citrus." *Biological Control* 53, no. 1: pp122-128, 2010.

[4] C. Dennis and J.Webster. "Antagonistic properties of species-groups of Trichoderma: III. Hyphal interaction". *Transactions of the British Mycological Society*, vol.*57*(3), pp.363-IN2,1971.

[5] M.A.G. Barbosa, K.G. Rehn, M. Menezes and R.D.L.R. Mariano. Antagonism of Trichoderma species on Cladosporium herbarum and their enzimatic characterization. *Brazilian Journal of Microbiology*, *32*(2), pp.98-104, 2001.

Financial assistances by National Science Foundation (NSF) under the research grant No RG/2016/AG/01 is acknowledged.

PREPARATION OF CHITOSAN NANOPARTICLES IMPREGNATED ACTIVATED *Eichhornia crassipes* (WATER HYACINTH) PETIOLE AS NOVEL SUSTAINED PLANT NUTRIENT DELIVERY SYSTEM

J.G. Shantha Siri[1]*, C.A.N. Fernando[1], S.N.T. De Silva[2], G.G. Chathuranga [1]

[1] *Department of Electronics, Faculty of Applied Sciences, Wayamba University of Sri Lanka, Kuliyapitiya.*

[2]*Department of Nano Science Technology, Faculty of Applied Sciences, Wayamba University of Sri Lanka, Kuliyapitiya.*

**Corresponding Author (email: shantha@nsf.ac.lk)*

Introduction

Nanotechnology involves creating and using structures, devices, and systems at the atomic, molecular, or macromolecular levels, 1-100 nanometer (nm) range at least in one dimension. Some recent research in nanotechnology has demonstrated promising perspective of nanofertilizer development and application. These nanomaterials are promising candidates of a new type of fertilizers (nanofertilizers) to meet the incoming challenges of food security and environmental protection. Compared with the conventional ones, nanofertilizers are expected to significantly improve crops growth and yields, enhance the efficiency of fertilizer use, reduce nutrient losses, and/or minimized. Research have been carried out to assess potential loading of NPK to chitosan nanoparticles to be used as a nanofertilizer for control release of plant nutrients. Majority of the polymers developed and available in the market are synthetic polymers and therefore, polymers used for encapsulation/coating nanofertilizers are also synthetic of which monomers will not be environmental friendly. There is a growing concern over the synthetic polymers due to environmental issues and importance of use of natural polymers has been highlighted.

Synthesis of eco-friendly nanoparticles has become a matter of great interest in recent years as it has diverse range of applications. Main aim of this study was to prepare macronutrient (NPK) loaded chitosan nanoparticles and to incorporate macronutrient loaded chitosan nanoparticles into the activated water hyacinth petiole as an environmentally friendly approach to develop sustained plant nutrient delivery system. Control release properties of the chitosan nanoparticles are enhanced further by incorporating it into activated water hyacinth petiole. Nanoparticle mediated sustained nutrient delivery systems have potential to enhance plant growth and yield. Since chitosan is a biodegradable, biocompatible and non-toxic biopolymer, unforeseen environmental, health and safety risks of

engineered nanomaterials are kept at a minimal. Sustained plant nutrient delivery systems also minimize serious environmental impacts resulted in excessive use of conventional bulk fertilizers.

Materials and Methods

In this study, chitosan nanoparticles were synthesized using ionotrophic gelation method with tripolyphosphate. Chitosan nanoparticles were sonicated with known concentrations of Potassium chloride, Calcium phosphate and Urea to load macronutrients. Natural precursor (water hyacinth petiole) was activated using chemical activation method with H_3PO_4. Macronutrient loaded chitosan nanoparticles were sonicated with activated fibers to incorporate it into the activated fibers. SEM images and EDX analysis indicated adsorption of chitosan nanoparticles into the activated fibers. Interaction of nutrients with chitosan nanoparticles were analysed with FTIR.

Analysis of nutrient release behavior

Normal loam soil samples were collected from the applied science faculty premises. pH of the collected soil samples was measured, known amount of soil was mixed with nutrient loaded chitosan nanoparticles impregnated water hyacinth placed in separatory funnels. This was carried out for Nitrogen (N), Phosphorous (P) and Potassium (K) separately. After placing the soil samples in separatory funnels, the funnels were filled with distilled water where water level was maintained just above the soil level to make sure that soil is saturated with water. Thereafter, water samples were collected from the separatory funnels weekly and stored for analysis of nutrient release behavior. After collecting the water samples at each stage, the funnels were replenished with distilled water to maintain the said level of water. Collection of water samples were carried out for a period of two months. Analysis of phosphate was carried out with Phosphomolybdate method.

Results obtained and discussion

Optimum pH for synthesizing chitosan nanoparticles was 5.0 and size of the particles found to be highly dependent on pH of the medium. FTIR spectra of nutrient loaded chitosan nanoparticles were compared with the FTIR spectrum of pure chitosan. A deviation of peaks corresponding to primary amine group and hydroxyl group in the chitosan molecules was observed (Figures 2 &3) which indicates interaction of macronutrients with the chitosan nanoparticles. In acidic medium, primary amine group get protonated and impart a positive charge to the chitosan molecule. Negatively charged nutrient ions (phosphate) can bind to the chitosan molecules by electrostatic interactions with the protonated anime group. Potassium is attracted to the

chitosan nanoparticles through electrostatic interaction with the hydroxyl group of chitosan nanoparticles. Since nanoparticles has a significantly increased surface area, it has capacity to interact with more and more nutrient ions. In addition to the electrostatic interactions, the nutrient ions are incorporated into the chitosan nanoparticles through the process of physical adsorption. FTIR spectra analysis indicates that nutrient is loaded into the chitosan nanoparticles through both mechanisms. Analysis of release kinetics (Figure 4) clearly indicates that nutrient is released for a period of two months. According to the literature on release kinetics pertaining to conventional bulk fertilizers, it has been shown that there is an initial burst and nutrient release stagnates after about 30 dates.

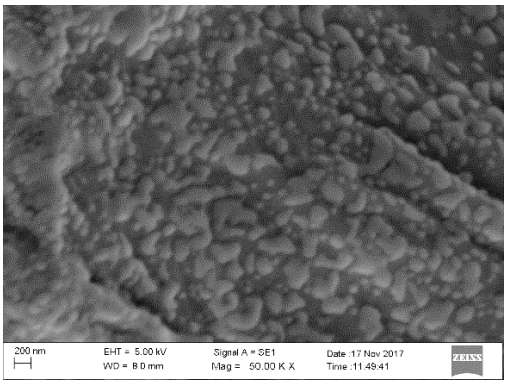


Figure 1. SEM Image of NPK loaded Chitosan nanoparticles.

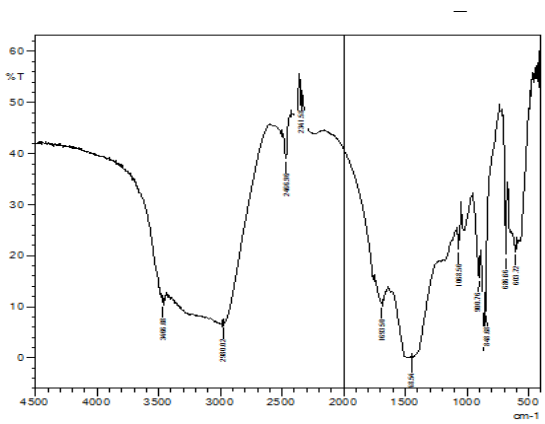


Figure 2. FTIR spectrum of potassium loaded chitosan nanoparticles

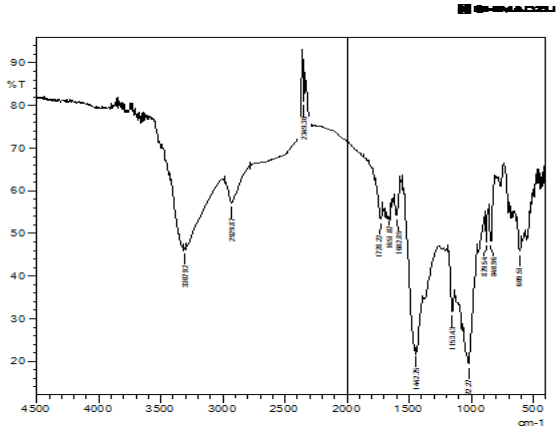


Figure 3. FTIR spectrum of phosphate loaded chitosan nanoparticles

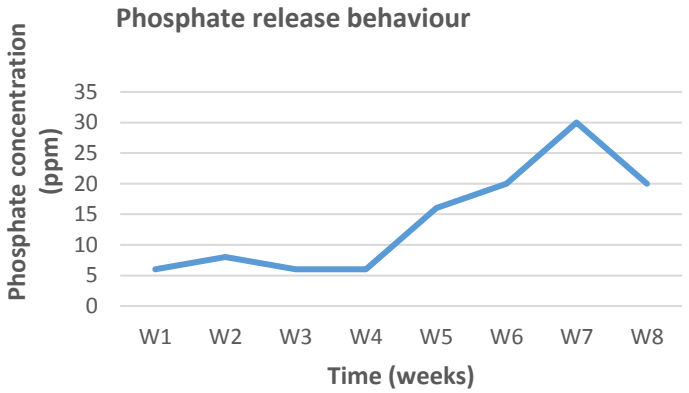


Figure 4. Phosphate release behavior of chitosan nanoparticle impregnated activated water hyacinth

Conclusions and Recommendations

Chitosan nanoparticles can be synthesized with simple ionotrophic gelation method with stringent control of pH in the medium and size of the particles depend of pH of the medium. According to the results obtained so far seems promising in relation to sustained release of nutrients and further replicate trials are in progress to confirm these results.

References

[1] Chen L., Xie Z.G., Zhuang X.L., Chen X.S., Jing X B.:Controlled release of urea encapsulated by starchg-poly (L-lactide). Carbohydrate Polymers, **72**, 342–348 (2008).

[2] Guclu G, Iyim T, Emik S, Ozgumu S,Synthesis and properties of starch-graft-

acrylic Acid / Na-montmorillonite superabsorbent composite hydrogels, *J Appl Polym Sci*, **109**, 16-22,20 08.

[3] Goel, A. Agricultural Applications of Nanotechnology *J. Biol. Chem. Research, 32 (1): 260- 266,2015*

[4] Liu M, Liang R, Zhan F, Liu Z, Niu A,Preparation of superabsorbent slow release nitrogen fertilizer by inverse suspension polymerization, *Polym Int*, **56**, 729-737, 2007

[5] Tarafdar, J.C., Agrawal, A., Raliya, R., Kumar, P., Burman, U. and Kaul, R.K. 2012b. ZnO nanoparticles induced synthesis of polysaccharides and phosphatases by *Aspergillus* fungi. *Adv*. *Sci*. *Engineering Med*., 4**:** 1-5.

[6] Tarafdar, J.C., Xiang, Y., Wang, W.N., Dong, Q. and Biswas, P. 2012c. Standardization of size, shape and concentration of nanoparticle for plant application. *Appl*. *Biol*. *Res*., 14**:** 138-144.

[7] Wu L, Liu M, Liang R, Preparation and properties of a double-coated slow-release NPK compound fertilizer with superabsorbent and waterretention, *Bioresource Tech*, **99**, 547-554, 2008.

POTENTIAL USE OF PLAIN TUBES FOR *In vitro* BLOOD GLUCOSE ANALYSIS; AN ECONOMICAL SAVING

N. I. Hewage*, D.Y.M.L. Dassanayake, D. I. Uluwaduge

Department of Allied Health Sciences, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka
**Corresponding author (email: ishiharinawoda@gmail.com)*

Introduction

Diabetes Mellitus (DM) is a common metabolic derangement debilitating populations in both affluent and less-affluent societies alike.It is characterized by a high blood sugar levels over a prolonged period. Blood glucose analysis is vital for both the diagnosis and monitoring of therapy in patients with DM. The current diagnostic limits of DM are narrow and therefore there is an increase need for reliable results to classify individuals correctly.

In the routine laboratory set up blood specimens for glucose determination have been collected in tubes that contain NaF (to prevent coagulation and glycolysis) and potassium oxalate ($K_2C_2O_4$) or EDTA disodium salt (Na_2EDTA, to prevent glucose utilization by red blood cells). In 1941, NaF containing tubes were introduced into laboratory practice for blood collection in determination of glucose [1].It is known that NaF shows its antiglycolytic effect by inhibiting the enolase enzyme of the glycolytic pathway in erythrocytes. However it has been recognized that the action of NaF towards enolase is slow as it will start at least four hours from the collection of blood [2]. Therefore, Al-Kharusi et al., and Fernandez et al., reported that there was no difference in blood glucose values in SST and NaF tubes which were separated within two hours of collection [1].The potential disadvantage of NaF tubes has been reviewed by the American Diabetic Association (ADA) laboratory guidelines for the diagnosis and management of DM with its recommendation to stop use of NaF as an antiglycolytic agent [3].

Further it has been pointed out by Fernandez et al.,that these tubes are useful if there is a several hour's delay in the separation of plasma from cellular components[2].However studies done by Chan et al., showed that following four hours, the concentration of glucose in whole blood in the presence of fluoride remains stable up to seventy two hours at room temperature and recommended the use of NaF if there is a delay in separation time [4].Considering the slow activity of NaF towards enolase enzyme, the use of NaF tubes to collect blood for this core biochemical test has to be revisited [2].

In turn, the specimens collected into these tubes are not suitable for the measurement of other key anylates such as Na+, K+ and enzymes and therefore multiple different collection containers are required.

In Sri Lankan laboratory set up, NaF containing tubes are recommended for collection of blood in analysis of glucose. The objective of this study was to compare glucose values obtained using plasma-NaF tubes and serum- plain tubes in a routine laboratory setup, and to assess the changes in glucose levels in plasma and serum up to six hours from the separation of cellular components. Collection of blood into plain tubes is more convenient in the point of patient caring if multiple tests has to be performed especially from critical care patients. On the contrary the use of the historical tubes (NaF as the preservative) was not revised though there is a great advancement of the technology today.

Materials and methods

The study was conducted at the central laboratory of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka during six months of period (from March to September 2017). Sixty one participants (healthy volunteers) were enrolled in the study after they provided written consent. The study was ethically approved by the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura (Protocol approval No.MLS/08/2017).

During the study period, blood samples (2.5 ml) were collected in to both plain and NaF/$K_2C_2O_4$ tubes. Following separation of plasma (NaF/$K_2C_2O_4$)NaF and serum(plain tube), glucose concentrations were measured in both plasma and serum by glucose oxidase method (DiaSysGOD FS reagent kit) usingShimadzu UV 1601 visible spectrophotometer (Simadzu Corporation, Kyoto, Japan).For both plasma (in NaF/$K_2C_2O_4$ tubes) andserum (in plain tube) samples, five glucose determinations were madebased on the serum separation time. Those five determinants were baseline glucose values and glucose concentration at one, two, four and six hours after serum or plasma separation. Samples which did not show haemolysis were included in the comparison.

The data are represented as mean±SD for continuous variables. A student's t-test was applied for comparison of group means. $p<0.05$ was considered statistically significant.

Results and Discussion

Glucose concentrations observed in plasma (NaF/$K_2C_2O_4$) and serum (plain tubes) upto six hours post separation and the percentage reduction in comparison with base line value is depicted in Table 1. Comparing plasma (NaF/$K_2C_2O_4$) and serum (plain tube) glucose values (n=61, paired t- test) showed no significant difference up to four hours of separation ($P>0.05$). In fact blood specimens collected into plain tubes are much better since it had shown minimal reduction up to two hours post separation. Nevertheless, glucose concentrations measured after four and six hours in plasma and serum showed a significant difference ($p< 0.001$).Furthermore a greater reduction was observed with serum glucose values

four and six hours post separation which the blood samples drawn into plain tubes. Blood collected to the NaF/ $K_2C_2O_4$ tubes showed a higher rate of hemolysis (11%) when compared that with the samples collected to plain tubes (4%).

Table 1: Mean glucose concentrations of plasma (NaF/$K_2C_2O_4$) and serum (plain tube) and percentage reduction of glucose values with respect to baseline; post separation up to six hours

	Plasma glucose (mmol/l) mean±SD	Percentage reduction in plasma	Serum glucose (mmol/l) mean±SD	Percentage reduction in serum
Baseline	6.3±2.7*	-	6.2±2.7*	-
1 hour	6.1±2.6*	3	6.2±2.7*	0
2 hour	5.9±2.6*	6.3	6.1±2.7*	1.6
4 hour	5.7±2.7**	9.5	5.0±2.6**	19
6 hour	5.6±2.6**	11.1	4.7±2.4**	24

*Mean glucose values in NaF/$K_2C_2O_4$and plain tubes are not significantly different ($p>0.05$).

**Mean glucose values in NaF/$K_2C_2O_4$and plain tubes are significantly different ($p<0.001$).

The Spearmen correlation between plasma (NaF/K2C2O4) and serum (SST) glucose concentrations up to four hours from the collection revealed a significant correlation approaching unity with R2=0.9990 (Figure 1).

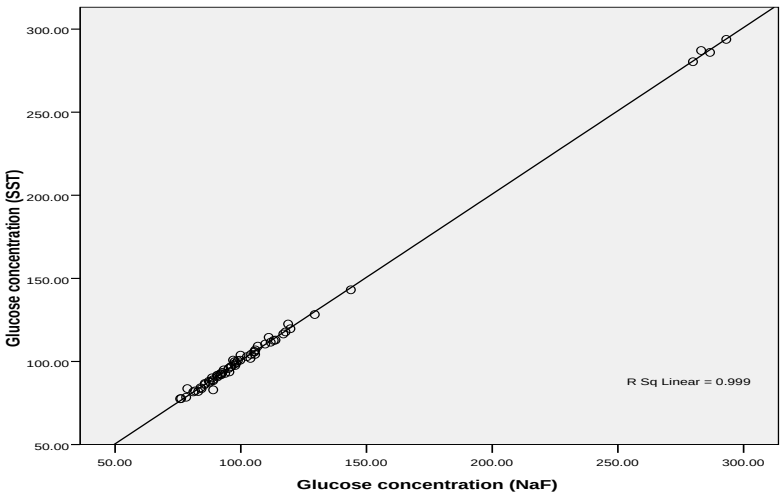


Figure 1. Spearmen correlation coefficient between plasma (NaF/$K_2C_2O_4$) and serum (SST) glucose concentrations up to four hours from the collection

The present study has confirmed that both plain tubes could be used in the palace of NaF/$K_2C_2O_4$ tubes to collect blood for glucose measurements with accepted values under routine laboratory setup. In both tubes clinically equivalent results yielded given that the blood sample processing had an optimum collection to separation time less than two hour.

These results are comparable with the findings by previous investigators [1, 4]. With the progress and improvements of the technology, it has been revealed that serum separated within reasonable time after the collection (less than two hours) gives same blood glucose values as in plasma in NaF tubes. The maximum separation time documented in American Diabetes Association (ADA) guidelines is thirty minutes from collection. A significant reduction in blood glucose values collected to NaF/$K_2C_2O_4$ have been observed by several workers and the difference reported were proportional to the time delay [2].The use of these historical tubes appeared to be suitable for blood collection if there is a long delay in the separation. According to Chan et al., NaF has a minimal preservative effect within two hours of blood collection [4]. Its effectiveness was reported after four hours of blood collection and activity persisted at least for three days at room temperature.

If NaF/$K_2C_2O_4$tubes are replaced with plain tubes, several laboratory tests including glucose could be done from a single vial. This is especially important with critical care and geriatric patients where there may be difficulties in bleeding. Additionally, use of plain tubes offer significant financial saving and this is more important in state sector hospitals of middle income countries such as Sri Lanka where the free health facilities are offered.

Further, it was observed that, the blood samples collected in NaF/ $K_2C_2O_4$ tubes had the higher rate of haemolysis (11%) when compared that with plain tubes (4%). Most of the studies have shown the similar results [1]. This could be due to the presence of larger crystals of NaF/ $K_2C_2O_4$which could rupture the RBC resulting the higher rate of haemolysis in those tubes [5].

Conclusions and Recommendations

Clinically acceptable blood glucose values could be obtained by using plain tubes instead of NaF/ $K_2C_2O_4$tubes if the separation is achieved within a reasonable time of less than two hours following blood collection. However, if blood separation is delayed or expected to be delayed for more than two hours following blood collection, fluoride containing tubes could be recommended for glucose measurement.

It is recommended to study the correlation in glucose values obtained to NaF/ $K_2C_2O_4$ tubes and plain tubes by using samples with high blood glucose values.

References

[1] A.Al-kharusi , N.Al-lawati , M.Al-kindi . "Are Tubes Containing Sodium Fluoride Still Needed for the Measurement of Blood Glucose in Hospital Laboratory Practice".*Oman Med J, 29(6),* pp.404–407.Nov. 2014.

[2] L .Fernandez , P.Jee, M Klein, P.Fischer, S.L.Perkins, S.P.J.Brooks. "A comparison of glucose concentration in paired specimens collected in serum separator and fluoride / potassium oxalate blood collection tubes under survey " field " conditions". *Clin. Biochem*. ol 46, Issues 4–5, pp.285-288. March 2013.

[3] D.B.Sacks, M. Arnold, G.L.Bakris, E.D.Burns, A.R.Horvath, M.S.Kirkman, A.Limmark,B.E.Metzger, D.M.Nathan. "Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus". *Clin.Chem*2011;57:6, pp.e1-e47.May.2011

[4] Chan AYW, Smaminathan R, Cockram CS."Effectiveness of sodium fluoride as apresevative of glucose in blood". *Clin Chem*, vol.35,pp.315-317. Feb.1989.

[5] Waring WS, Evans LE, Kirkpatrick CT. "Glycolysis inhibitors negatively bias blood glucose measurements: Potential impact on the reported prevalence of diabetes mellitus".*Jof Clin Path. vol 60. June.2007.*

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACT AND ITS SOLVENT PARTITIONED FRACTIONS OF SRI LANKAN MARINE RED ALGAE *Gracillaria edulis* (Gmelin) Silva

M.D.T.L. Gunathilaka[1], K.W. Samarakoon[3], P. Ranasinghe[2], D. Peiris[1]*

[1]*Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda*
[2]*Industrial Technology Institute, Halbarawa Gardens, Malabe*
[3]*National Science and Technology Commission, Dudley Senanayake Mawatha, Colombo*
**Corresponding author (email:dinithi@sci.sjp.ac.lk)*

Introduction

Among the under-explored marine flora, seaweeds are an important source of bioactive metabolites in drug development and nutraceuticals. Bioactive compounds are generated as a result of their biochemical and physiological mechanisms. Some of these bioactive compounds are used in pharmaceutical industries for various therapeutic purposes as they exhibit anti-oxidant, anti-diabetic, anti-proliferative, cytotoxic and anti-inflammatory properties. Oxidative stress plays a major role in chronic inflammation and it is tightly linked with the pathophysiological processes of numerous degenerative diseases such as diabetes, cardiovascular disorders, cancer, inflammation and aging. Therefore, in lieu with herbal drug development, combat of oxidative stress through natural antioxidants present in less utilized marine flora are very important since there are under explored in Sri Lanka (Correa-Rotter *et al.,* 2004).

Gracillaria edulis (Gmelin) Silva is a red algae belongs to the family Gracilariaceae. Polyphenols purified from red algae are considered as a rich source of antioxidants with significant health promoting properties (Correa-Rotter *et al.,* 2004). Therefore, the present study was undertaken to investigate the phytochemicals and in-vitro antioxidant potential of methanolic extract and its solvent fractions of *G.edulis.*

Materials and Methods

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

Homogenized powder (10.0g) extracted three times using 70% methanol was subjected to sonication at 25 °C for three 90 min periods. The polyphenols were separated by precipitating crude polysaccharides by adding 25 volumes of 70% ethanol (v/w% -1:25) and allowed to stand overnight. The supernatant was

separated by centrifugation (12,000 rpm). The portion of the supernatant was used to solvent-solvent partition with hexane, chloroform and ethyl acetate respectively (Lakmal *et al.,* 2014). The resulting hexane fraction (GEMH), chloroform fraction (GEMC), ethyl acetate fraction (GEME), aqueous fraction (GEMA) and crude methanol extract (GEMM) of *G.edulis* were used for qualitative and quantitative determination of phytochemicals and for evaluation of antioxidant activities.

Methanol extract of *G. edulis* was used for qualitative analysis of phytochemicals. Cardiac glycosides, saponins, terpenoids, phenols, alkaloids, steroids, tannin and phytosterols were analyzed by qualitative chemical methods (Jeyaseelan *et al.,* 2012). Total polyphenolic and total flavonoid content were determined using Folin-Ciocalteu (Singleton *et al*., 1999) and aluminum chloride method (Chang *et al.,* 2002) respectively. Total alkaloid content was determined by Sreevidya *et al*., (2003) with some modifications.

Four different in-vitro antioxidant assays were used to determine the antioxidant activity of methanolic extract and fractions of *G.edulis.* Ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity was performed according to the method described by Benzie & Szeto (1999) and Blois (1958) respectively. The ferrous iron chelating capacity (FICC) and oxygen radical absorbance capacity (ORAC) was determined using ferrozine reagent according to the method by Carter (1971) and Ou *et al.,* respectively (2001).

Statistical analysis was performed using Minitab 2017 and Excel 2013. All data values are expressed as mean±standard deviation based on three replicates. P values less than 0.05 ($P< 0.05$) was considered as significant.

Results and Discussion

The qualitative phytochemical screening revealed the presence of cardiac glycosides, saponins, terpenoids, phenols, alkaloids, steroids, tannin and phytosterols in the methanolic extract of *G. edulis.* The results of quantitative analysis of TPC, TFC and total alkaloid content is presented in table 1.

Results showed the significant difference of TPC in four different fractions ($P<0.05$). The highest TPC and TFC were reported in GEME fraction (2414.51±50.34 µg Gallic acid equivalent/g of fraction and 1461.49±75.22 µg quercetin equivalents/g of fraction) when compared to crude methanolic extract (1007.81±54.21 µg Gallic acid equivalent/g of extract and 541.01±51.84 µg quercetin equivalents/g of fraction). The highest alkaloid content was observed in crude methanolic extract (173.41±4.03 mg alkaloids/g of extract) of *G.edulis* compared to four fractions. Ganesan *et al*., (2008) reported that the total phenolic content of ethyl acetate fraction of *G.edulis* as 7.81± 0.76 mg gallic acid equivalents/g extract. The present study showed comparatively lower content of TPC than the previous study compared. This might be due to the environmental

variations which they are grown.

Table 01. phytochemical analysis of methanolic extract and fractions of *G.edulis*

	TPC (µg gallic acid equivalents/g of extract)	TFC (µg quercetin equivalents/g of extract)	Alkaloids mg of alkaloids/ g of extract
Methanol extract (GEMM)	1007.81±54.21	541.02±51.84	173.41±4.03
Hexane fraction(GEMH)	760.85±37.75	688.60±9.55	60.96±5.45
Chloroform fraction(GEMC)	560.85±55.08	289.39±9.55	58.40±5.26
Ethyl acetate fraction (GEME)	2414.51±50.34	1461.49±75.22	65.01±5.78
Aqueous fraction (GEMA)	1704.69±43.16	786.95±62.04	141.84±19.27

Results represent means ± standard deviation of triplicate determinations.

The antioxidant potential of *G. edulis* was measured by its ability to scavenge the stable DPPH radical (Table 02). The highest DPPH radical scavenging activity was reported in GEME fraction of *G.edulis* (2732.81±36.49 µg Trolox equivalents/g of extract) with IC_{50} of 3.17±0.04 mg/ml compared to standard trolox (IC_{50}:8.68±0.06 µg/ml). Present study revealed that the reduction of DPPH occurred in a concentration-dependent manner as observed with the high reduction of DPPH (higher radical activity) in high concentrations (table 03).

According to study conducted by Ganesan *et al.,* (2008) in India using methanol extract and fractions of *G. edulis*, ethyl acetate fraction has shown 4.73% DPPH radical scavenging activity at 1000 µg/ml and methanol extract has shown 5.20% DPPH scavenging activity at 1000 µg/ml. When compared to present study, 21.06% and 19.52% DPPH radical scavenging activity was observed in GEME fraction and methanol extract of *G.edulis* at 937.5 µg/ml respectively.

Francavilla *et al.*, (2013) reported the biochemical composition of *G.edulis* vary with seasonal factors. According to the study of Francavilla *et al*, EA fraction of the sample collected in July showed highest DPPH activity (EC_{50}:0.82 mg/ml) than the EA fraction of another sample collected in October (EC_{50}:2.55 mg/ml). Present study reported 3.17mg/ml IC_{50} for EA fraction of *G.edulis* which was collected in February. Differences in activity may be due to the seasonal factors.

Table 02. Antioxidant activity of methanolic extract and fractions of *G. edulis*

	Antioxidant activity			
	DPPH (µg Trolox equivalents/g of extract)	**FRAP (µg Trolox equivalents/g of extract)**	**FICA (µg EDTA equivalents/g of extract)**	**ORAC (µg Trolox equivalents/g of extract)**
GEMM	2720.36±22.18	268.95±34.97	2093.32±36.75	1446.36±56.19
GEMH	1394.76±2.19	1661.75±60.81	7552.42±95.89	531.43±25.18
GEMC	2634.82±18.61	2405.35±14.41	8022.24±48.60	770.84±48.04
GEME	2732.81±36.49	8505.50±44.27	8750.27±33.21	1462.88±16.39
GEMA	2220.64±14.36	1417.10±41.38	7186.33±43.41	363.93±27.52

Data represented as mean ± SD of triplicate determinations. GEEMM- *Gracillaria edulis* 70% methanol extract; GEMH- *Gracillaria edulis* 70% methanol extract hexane fraction; GEMC- *Gracillaria edulis* 70% methanol extract chloroform fraction; *GEME- Gracillaria edulis* 70% methanol extract ethyl acetate fraction; GEMA- *Gracillaria edulis* 70% methanol extract aqueous fraction

Table 3. Dose response relationship for DPPH radical scavenging activity

	(% Inhibition)						
	Assay Concentration (mg/ml)						
	3.75	**1.875**	**0.938**	**0.469**	**0.235**	**0.117**	**IC_{50}**
GEMM	55.38±0.5	35.83±0.4	21.05±0.2	13.41±0.4	11.01±0.7	6.98±0.4	3.19±0.02
GEMH	56.43±0.2	36.66± 0.6	23.70± 0.3	14.85±0.9	11.89±0.2	9.57±0.5	6.22±0.01
GEME	57.95±0.5	31.06±0.3	19.52±2.4	16.07±0.5	11.41±0.1	3.74±0.7	3.17±0.04
GEMC	54.68±0.4	32.85± 1.2	20.95± 0.4	15.29±0.3	9.81±0.7	4.79±0.5	3.29±0.02
GEMA	46.92±0.3	29.45±0.5	18.25±0.7	13.46±0.2	9.45±0.4	4.96±0.2	3.90±0.02

Data represented as mean ± SD of triplicate determinations. GEEMM- *Gracillaria edulis* 70% methanol extract; GEMH- *Gracillaria edulis* 70% methanol extract hexane fraction; GEMC- *Gracillaria edulis* 70% methanol extract chloroform fraction; *GEME- Gracillaria edulis* 70% methanol extract ethyl acetate fraction; GEMA- *Gracillaria edulis 70%* methanol extract aqueous fraction.

The ferric reducing antioxidant power (FRAP) assay uses antioxidants as reductants. Hence, the antioxidants present in the plant extract can reduce the Fe3+/ferricyanide complex to the ferrous form (Sampath *et al.,* 2015). In the present study, highest FRAP was observed in GEME fraction (8505.50±44.27 µg Trolox equivalents/g of extract). In contrast, Francavilla *et al.*, (2013) had reported highest reducing power in ethyl acetate fraction (808.90 µmol Trolox/ g of extract) of *G.edulis* collected seasonally in the Lesina Lagoon at Italy during the period of July. The present study result shows higher reducing power in ethyl acetate fraction which was collected in February. Therefore differences in reducing power might be due to the seasonal variation.

The ferrous iron chelating capacity of *G.edulis* was determined using ferrozine reagent. Ferrozine can chelate with Fe^{2+} and form ferrozine-fe^{2+} red coloured complex (Ranasinghe *et al.,* 2012). The highest iron chelating activity was observed in GEME fraction (8750.27±33.21 µg EDTA equivalents/g of extract) with IC_{50} value of 2.22± 0.01 mg/ml compared to the standard EDTA (IC_{50}:19.34± 0.07 µg/ml). The % chelating activity was observed as 92.99±0.29% to the highest sample concentration (15mg/ml) and 13.97±0.37% for the lowest sample concentration (0.468mg/ml) respectively.

Oxygen radical absorbance capacity of methanolic extract and fractions of *G. edulis* is given in Table 02. The ORAC assay measures the oxidative degradation of fluorescein molecule. During ORAC Assay, peroxyl radicals are generated from the thermal decomposition of AAPH. In the presence of peroxyl radicals, fluorescein start to decay. Addition of samples rich in antioxidants reduce the decay of fluorescein molecule (Ganske, 2010). In the present study, highest Oxygen radical absorbance capacity was reported in methanolic extract (1462.88±16.39 µg Trolox equivalents/g of extract) of *G.edulis* compared to its solvent fraction.

Conclusions

It is concluded that methanol extract and its fractions of *Gracillaria edulis* contains phytochemicals and antioxidant activities with varying degrees of potential. However, EA fraction of *G.edulis* showed significantly high levels of bioactive phytochemicals and possess marked antioxidant activity. Hence the isolation of active compounds in ethyl acetate fraction is warranted.

References

[1] M.S. Blois. “Antioxidant determination by use of stable free radical”. *Nature,* vol. 181, pp. 1199-120, 1958.

[2] I.F.F. Benzie, Y.T. Szeto. “Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay”. *Journal of Agricultural and Food Chemistry*, vol. 47 (2), pp. 633-636, 1999.

[3] P. Carter. “Spectrophotometric determination of serum iron at the sub-microgram level with a new reagent ferrozine. *Annual Biochem*, vol. 40, pp. 450-458, 1971.

[4] R. Correa-Rotter, S. Naicker, I.J.Katz. “Demographic and epidemiologic transition in the developing world: role of albuminuria in the early diagnosis and prevention of renal and cardiovascular disease”. *Kidney Int.* vol. 66, pp. 32–37, 2004.

[5] E.C. Jeyaseelan, S. Kothai, R. Kavitha, S. Tharmila. “Antibacterial activity of some selected algae present in the costal lines of Jaffna Peninsula”. *International Jour.* 2012.

[6] V.L.Singleton, R. Orthofer, and R.M. Lamuela-Raventos. “Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocaltue reagent”. *Methods in Enzymol*. vol. 299, pp. 152-178, 1999.
[7] C.C.Chang, M.H.Yang, H.M.Ucen, J.C. Chern. “Estimation of total flavonoid content in propolis by complementary colorimetric method”. *Journals of food and drug analysis*. vol. 10, pp. 178-182, 2002.
[8] N. Sreevidya and S. Mehrotra. “Spectrometric method for estimation of precipitable with Dragendorff’s reagent in plant material”, *Journal of AOAC International*, vol. 36(6), pp. 1124–1127, 2003.
[9] Ganesan, P., Kumar, C. S. and Bhaskar, N. (2008) ‘Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds’, *Bioresource Technology*, 99(8), pp. 2717–2723. doi: 10.1016/j.biortech.2007.07.005.
[10]Francavilla, M. *et al.* (2013) ‘The red seaweed gracilaria gracilis as a multi products source’, *Marine Drugs*, 11(10), pp. 3754–3776. doi: 10.3390/md11103754.

Acknowledgement
Financial assistances under the research grant ASP/01/RE/SCI/2017/50.

ACUTE, SUB-ACUTE AND SUB-CHRONIC TOXICITY STUDY OF *Psychotria sarmentosa* LEAVES USED IN TRADITIONAL PORRIDGE IN SRI LANKA

W.M.K.M. Ratnayake[1], T.S. Suresh[1], A.M. Abeysekera[2], N. Salim[3], U.G. Chandrika[1]

[1]Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.
[2]Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.
[3]Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.
**Corresponding author (email: sugandhika@sjp.ac.lk)*

Introduction

Psychotria sarmentosa, generally known as ":*Gonica*" in Sinhala, is a climber of the Rubiaceae family. Leaves and stems of this plant are used to treat bone fractures, in the *Deshiya Chikista* system of medicine in Sri Lanka. Males in rural areas in Sri Lanka drink an aqueous extract of leaves and stems of this plant, when they are physically assaulted [1]. In addition, immature leaves are widely used by the community make a traditional leafy porridge ("*kola kenda*") and a tempered vegetable salad. Our previous studies have shown that freeze dried aqueous extract of leaves of this plant has significant ($p < 0.05$) acute and chronic anti-inflammatory activity in *in-vivo* models. 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 cyclooxygenase-2 and prostaglandin E_2 inhibitory activity. Although, it shows different biological actions no scientific data are available regarding its potential adverse effects. The insufficiency 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, sub-acute and sub-chronic toxicity of *P. sarmentosa* leaves.

Material and Methods

Plant material

Fresh *P. sarmentosa*, stems with leaves were purchased from a local market and authenticated by Dr. D. S. A. Wijesundara, Director General, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (KMR001) 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 aqueous extract of P. sarmentosa leaves

Fresh leaves of *P. sarmentosa* (100 g) were crushed in a mortar and pestle with 200 mL of water. The extract was filtered. The greenish filtrate was boiled for 5 minutes and cooled and then freeze dried. The extract yielded 2.4 g of green coloured freeze dried powder. The required amounts of freeze dried aqueous extract of *P. sarmentosa* leaves (FAPL) were dissolved in distilled water for oral administration to the rats.

Toxicity study of P. sarmentosa leaves

To evaluate the safety of the aqueous extract of *P. sarmentosa* leaves, a limited dose acute oral toxicity study, sub-acute oral toxicity study (28 days) and sub-chronic oral toxicity study (90 days) were carried out in compliance with the Organization for Economic Co-operation and Development (OECD) guidelines. In each assay negative control group was administered with 1.0 mL of distilled water (DW). In the limited dose test, treated group received 5000 mg/kg b. w. of FAPL. The sub-acute toxicity was done for the therapeutic effective dose of FAPL as found in anti-inflammatory assays (100 mg/kg b. w.) and doses which are lesser and higher than this dose, i.e.: 50 mg/kg b. w. and 2000 mg/kg b. w. respectively for 28 days. The sub-chronic toxicity study was done only for the therapeutically effective dose. In sub chronic assay recovery groups were kept for further 28 days without any oral treatments. 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 consumption and food consumption were compared with the control group. In addition to this haematological and biochemical parameters were measured to evaluate the safety of the plant extract. Further relative organ index was calculated to assess the safety of FAPL on different organs. Histopathological studies were also done.

Results and Discussion

All studies have shown that there were no mortalities during the entire period of the study following administration of FAPL. As mortality is the main criteria in

assessing the acute toxicity of any drug, the absence of mortality by FAPL 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 diarrhea, damaged skin, subcutaneous swelling or lumps, wetness or soiling of perineum, salivation and breathing abnormalities in treated group in comparison to the control group indicated that FAPL in acute, sub-acute and sub-chronic dosing.

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 in 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 FAPL 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 FAPL. Further, the results showed that, there are no significant ($p > 0.05$) difference in food and water consumption in treated groups as compared to negative control.

When considering the serum clinical profiles, all tested parameters in the rats treated with FAPL comparable with those of the control rats of each study. The results on sub-chronic study are given in Table 3.1. 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 FAPL treated group indicates its safeness on liver. Further, absence of any significant difference ($p > 0.05$) in serum urea and creatinine levels in FAPL treated rat group as compared to the control group indicate its safeness on kidney. The absence of difference in relative weights of vital organs in treated as compared to the control group also provide 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 also measured and any abnormal haematological parameters levels were not found in FAPL treated groups. The results on sub-chronic study are given in Table 3.2.

As histological assessment is also important in toxicology studies the collected tissues were subjected for histopathology observations include leukocyte infiltration, necrosis in liver, hemorrhages in kidneys and many others in each toxicity study. The results have shown that there were no any morphological changes in microscopic examination of tissues. Hence, it also provide strong evidence for the non-toxicity of FAPL.

Table 1. Clinical chemistry data of Wistar rats in the sub-chronic oral toxicity study of *P. sarmentosa* leaves

Clinical chemistry parameters	Group 1	Group 2	Group 3	Group 4
Albumin (mg/dL)	4.4 ± 0.1	4.3 ± 0.1	4.5 ± 0.1	4.4 ± 0.04
ALP (IU/L)	94.3 ± 16.6	113.8 ± 2.0	110 ± 2.8	108 ± 3.0
ALT (IU/L)	42.0 ± 2.3	43.6 ± 5.0	42.6 ± 2.5	45.8 ± 2.8
AST (IU/L)	78.2 ± 2.3	73.0 ± 3.1	79.1 ± 3.1	73.6 ± 2.4
Calcium (mg/dL)	10.8 ± 0.3	11.8 ± 0.3	11.1 ± 0.3	11.7 ± 0.6
Cholesterol (mg/dL)	72.3 ± 3.0	68.0 ± 1.8	73.2 ± 3.4	75.0 ± 3.6
Creatinine (mg/dL)	0.6 ± 0.02	0.7 ± 0.04	0.6 ± 0.04	0.6 ± 0.04
γ – GT (IU/ L)	22.2 ± 0.9	22.4 ± 0.9	22.4 ± 1.1	24.1 ± 0.9
Glucose (mg/dL)	83.7 ± 2.0	73.8 ± 5.3	75.5 ± 1.2	83.4 ± 1.3
Phosphorous(mg/dL)	18.3 ± 1.4	20.7 ± 1.4	19.0 ± 2.2	19.3 ± 1.3
Total bilirubin (mg/dL)	2.8 ± 0.2	3.0 ± 0.3	2.7 ± 0.2	3.2 ± 0.3
Urea (mg/dL)	3.8 ± 0.3	3.9 ± 0.5	3.4 ± 0.3	3.4 ± 0.1

Values for clinical chemistry parameters are expressed as mean ± SEM, (n=6/group), $^{*}p<0.05$ compared with healthy control

Group 1: Healthy control group (Distilled water), Group 2: Treated group (100 mg/kg b. w., FAPL in DW), Group 3: Healthy control recovery group (DW), Group 4: FAPL Treated recovery group (100 mg/kg b. w., FAPL in DW)

Table 2. Haematological parameters in Wistar rats of the sub-chronic oral toxicity study of *P. sarmentosa* leaves

parameters	Group 1	Group 2	Group 3	Group 4
Haemoglobin (g/dL)	14.9 ± 0.4	15.0 ± 0.4	15.0 ± 0.4	14.7 ± 0.2
RBC (x 10^9 / L)	7.6 ± 0.8	7.7 ± 0.1	7.9 ± 0.1	8.0 ± 0.1
PCV (%)	42.5 ± 1.2	43.7 ± 0.9	43.7 ± 0.8	43.1 ± 0.9
Platelet count (x10^9/L)	863 ± 55	847 ± 67	822 ± 43	848 ± 69
MCV (fL)	111 ± 1.5	113.5 ± 0.5	113 ± 1.2	108.6 ±1.7
MCH (pg)	39.0 ± 0.6	38.9 ± 0.4	40.0 ± 0.4	37.1 ± 0.5
MCHC (g/ dL)	70.2 ± 0.3	68.8 ± 0.7	70.8 ± 0.3	68.4 ± 0.4
WBC (x 10^9 / L)	6.7 ± 0.4	8.5 ± 0.6	6.8 ± 0.4	8.2 ± 0.4
Granules (x 10^9 / L)	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.2	0.9 ± 0.3
Lymphocytes(x10^9/ L)	5.4 ± 0.2	6.9 ± 0.5	5.5 ± 0.2	6.4 ± 0.6
Monocytes(x 10^9 / L)	0.8 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.1

Values for haematological parameters are expressed as mean ± SEM, (n=6/group), $^{*}p<0.05$ compared with healthy control

Group 1: Healthy control group (Distilled water), Group 2: Treated group (100 mg/kg b. w., FAPL in DW), Group 3: Healthy control recovery group (DW), Group 4: FAPL Treated recovery group (100 mg/kg b. w., FAPL in DW)

Conclusion

As the aqueous extract of *P. sarmentosa* leaves showed absence of acute, sub-acute and sub-chronic toxicity in Wistar rats, it is safe in using of short term as well as long term consumption. As aqueous extract of *P. sarmentosa* leaves has anti-inflammatory properties, it can be recommended as a safe treatment for chronic inflammatory disease conditions such as arthritis.

References

[1] Ratnasooriya, W. D, Dharmasiri, M. G (1999) A water extract of leaves and stems of *Psychotria sarmentosa* has analgesic and antihyperalgesic activity in rats, *Medical Science Research*, 27: 715 – 718.

[2] Allan, J. J., Bhide, R. M., Agarwal, A. (2012) Safety assessment of Zigbir ^{R}: A polyherbal formulation in Sprague-Dawley rats, *Journal of Toxicology,* volume 2012, Article ID 589520, http://dx.doi.org/10.1155/2012/589520

Acknowledgement

Financial assistance by World Class University Project (WCUP) under the research grant number Ph. D. /15/ 2012 is gratefully acknowledged.

PROXIMATE COMPOSITION OF DIFFERENTLY PROCESSED DIK WEE AND DAHANALA

T.P.A.U. Thennakoon* and S. Ekanayake
Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura
**Corresponding author (email:anuradithennakoon@gmail.com)*

Introduction

Rice (*Oryza sativa* .L) is the world's second largely produced cereal and is the staple food in most of the Asian countries including Sri Lanka [1]. Hence it affects the nutritional status of the whole population in our country. Among Sri Lankans the prevalence of non-communicable diseases such as diabetes and cardio vascular diseases has increased in the past years. A positive relationship between consumption of highly refined white rice and diabetes has been found [2]. Both the quality and the quantity of starch present in rice need to be considered when consuming. Presence of chemical compounds such as pigments which can act as antioxidants and blood sugar lowering agents and dietary fiber can make some differently processed rice varieties healthier. Loss of nutrients resulting from milling and polishing rice is very considerable as degree of milling and polishing determines the amount of nutrients removed. It had been estimated that in the process of milling brown rice to white rice approximately 80% of thiamin is removed [3]. Other nutrients contained in the bran layer are also lost, including niacin, iron and riboflavin.

However, no sufficient data is available regarding the proximate composition of differently processed traditional rice varieties in Sri Lanka. Thus the main aim of this study was to investigate the effect of processing on proximate composition of Dahanala and Dik wee rice varieties.

Methodology

Selection and processing of rice varieties:

Raw paddy was collected from the traditional rice preserving centre at Homagama. Paddy was parboiled by immersing paddy in boiling in hot water and heating until the paddy grains split open and sun drying. Milling and polishing of rice was done at Industrial Technology Institute. Two traditional rice varieties were differently processed as raw under milled, raw polished (4% polishing level) and parboiled under milled.

Determination of proximate composition:

Proximate composition (moisture, mineral (as ash), digestible starch, protein and fat) determination of the selected rice varieties were carriedout using standard methods. Each rice sample was cooked (as per home cooking), oven dried at low

temperature (40-45 ^{o}C) and milled and seived (100 mesh size). The flour thus produced was used for below mentioned analyses.

- Moisture and ash content (AOAC method)
  The samples(n=5) were oven dried at 105 ^{o}C until constant weight using porcelain crucibles for moisture determination and samples were ignited in the muffle furnace at 550 ^{o}C for 5-6 hours for ash determination.

- Digestible Starch
  Samples were incubated at 100°C with α-amylase followed by amyloglucosidase at 50 °C and starch content assayed as glucose following reaction with glucose oxidase colorimetrically. (Megazyme starch assay kit)
- Crude Protein (Kjeldhal method)
  Micro Kjeldhal apparatus was used and the coversion factor for rice used was 5.75.
- Fat content (Croon *et al*., 1980)
  Samples were digested with 7.7M HCl and fat was extracted with petroleum ether and diethyl ether using majoiner flasks.

SPSS statistic package was used in data analysis. (Descriptive statistics and ANOVA Tukey's posthoc test at 95% confidence interval)

Results and Discussion

Proximate composition of cooked rice varieties on wet weight basis is shown in the table 1 below.

Table 1: Proximate composition of cooked rice varieties on wet weight basis (g/100g) (mean±SD)

Composition	Dik wee			Dahanala		
	Raw	Raw polished	Parboiled	Raw	Raw polished	Parboiled
Moisture*	68.7±1.8^{a}	65.7±1.7^{a}	84.5±6.4^{b}	66.0±2.1^{a}	64.9±0.4^{a}	83.6±2.8^{b}
Ash*	0.39±0.01^{a}	0.29±0.01^{b}	0.15±0.01^{c}	0.42±0.03^{ad}	0.29±0.01^{be}	0.20±0.01^{f}
Digestible starch*	24.0±0.3^{a}	31.0±1.7^{b}	11.5±0.2^{c}	23.4±1.1^{a}	28.3±0.7^{d}	12.1±0.1^{c}
Fat*	1.3±0.1^{a}	0.9±0.1^{b}	0.7±0.1^{c}	1.4±0.1^{d}	1.5±0.1^{d}	0.7±0.1^{c}
Protein**	2.8±0.1^{a}	1.9±0.2^{b}	1.6±0.1^{b}	3.7±0.1^{c}	3.5±0.1^{c}	1.9±0.1^{b}

a-f indicate the significant difference at $p<0.05$ in each row within and between varieties ; *n=5, **n=3

Moisture contents of both parboiled Dik wee and Dahanala varieties were significantly higher ($p<0.05$) than raw and raw polished varieties. Ash contents of raw rice were higher compared to raw polished and parboiled but were less than 0.5g/100g of cooked rice. Digestible starch contents of raw, raw polished and parboiled varieties of both Dik wee and Dahanala were significantly different ($p<0.05$) to each other. Raw polished had significantly high ($p<0.05$) digestible starch content compared to parboiled and under milled raw rice. This is due to

the increased moisture retention in parboiled rice due to parboiling process and removal of rice bran in raw polished rice during the milling process. Fat and protein contents of both parboiled varieties were significantly lower due to the higher moisture content in parboiled varieties. Dahanala had higher protein content than Dik wee irrespective of the processing. However,Dik wee raw had significantly high fat and protein contents compared to raw polished variety.

Conclusions

When considering parboiled rice the amount of digestible starch per 100g fresh weight was less compared to raw and raw polished rice due to high moisture. By consuming parboiled rice, intake of digestible starch can be lowered without affecting other nutrient content in rice compared to raw polished rice. Fat and protein contents of raw under milled varieties were higher than raw polished and parboiled varieties. When considering the raw under milled and polished rice, under milled rice contained more fat and protein.

References

[1] Abesiriwardana D.S.De.Z., 2003, “Rice varietal improvement for future challenges”, in Rice congress 2000: Future of rice in Sri Lanka. Pp.87-97

[2] Jenkins DJA, Wolever TMS, Taylor RH, Barker H, Fielden SRN, Baldwin JM, *et al.* Glycaemic index of food: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition* 1981; **34**: 362-6.

[3] Heinemann R, Fagundes P, Pinto E, Penteado M, Lanfer-Marquez U. Comparative study of nutrient composition of commercial brown, parboiled and milled rice from Brazil. *J Food Comp Analy.* 2005; **18(4):**287-296.

MECHANOCHEMICAL SYNTHESIS OF CITRONELLA OIL ENCAPSULATED MONTMORILLONITE NANOCOMPOSITE AS A MOSQUITO REPELLING AGENT

S. Ramanayaka*[1, 2], A. Hulangamuwa[3], N.M. Adassooriya[1], J. Walpita[1], M. Vithanage[1]

[1]*Ecosphere Resilience Research Center, Faculty of Applied sciences, University of Sri Jayewardenepura, Sri Lanka*

[2]*Postgraduate Institute of Science, University of Peradeniya, Sri Lanka*

[3]*The Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka*

**Corresponding author (email: dsammaniramanayaka@gmail.com)*

Introduction

Increasing population of mosquitoes are difficult to control due to their population density, reproductive capacity, genomic flexibility and metamorphosis [1]. Therefore, to control these blood sucking insects, scientists have developed environmental friendly control methods, such as Insects repellents, which is one of the most popular insect controlling methods in the world. Developing a repellent is a challenge because of the poorly known physiological mechanism of repellents, undefined different repellent phenomena and testing and quantifying the repellency [2]. Under the concept of green chemistry, natural insect repellents are more favored over chemically synthesized repellents. A repellent is a single or a mixture of volatile organic compounds that actively moves the responder out of the exact position to the opposite direction and reduces the attraction of insect until odor is faded [1]. In this study we have focused on repellents that repels insects, from odor source without direct contact.

Plant based insect repellents are known as minimum hazardous pesticides. They are essential oils, a mixture of volatile organic compounds and released under the suppressed conditions and more importantly evaporates at the room temperature. These natural products have a defense mechanism against insects and production of secondary metabolites, such as monoterpenes [4]. Most important insect repelling essential oils contain monoterpenes as their major component. In this study we used Citronella oil, which is commercially available, as the mosquito repellent essential oil [4]. Citronella oil contains constituents of citronellol, citronellal, geraniol, citral, α-pinene and limonene, which is monoterpenes, has the mosquito repellent activity. It is obtained from the leaves and stems of different species of *Cymbopogon nardus* (lemongrass) [3]. However, the issue facing with the repellents is that the release is fast and hence the period of activity is short.

This study intend to encapsulate citronella oil with a clay (montmorillonite) (Figure 1) which may formulate a clay-citronella composite that can slowly release the constituents of the essential oil to the environment for a considerably longer time period, with the repellent action. Montmorillonite (MMT) is a nanoclay which consist of two fused siloxane tetrahedral sheets sandwiching an edge-shared octahedral sheet, which is made up of aluminium or magnesium hydroxide. The objective of the study is to trap citronella oil between the regular gaps within two layers of MMT, which may result a long release time.

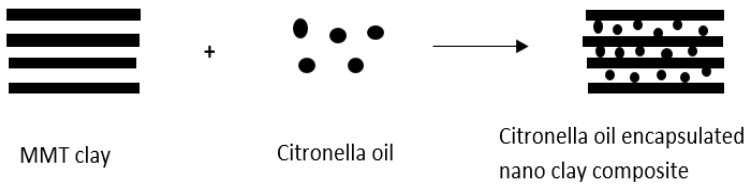


Figure1. Citronella oil encapsulated MMT nano clay composite

Materials and Methods

Preparation of clay nanocomposite

Commercially available citronella oil was used as the essential oil, which has the mosquito repellent activity and MMT (Southern clay company, USA) was used as the clay based material. A series of clay composite was prepared by adding 10 - 25% of citronella oil with MMT. Both compounds were ground together by using a mortar and pestle for about 3 hrs. With the mechanical force, essential oil entraps between the layers of clay material and form citronella oil encapsulated nanoclay.

Characterization of nanocomposite

The synthesized nanoclay compound was characterized with Powder x-ray diffraction (PXRD) to compare and contrast the interlayer spacing to observe the intercalation of citronella oil in MMT layers. X-ray diffraction patterns of all synthesized samples were recorded by using Rigaku Ultima IV x-ray powder diffractometer with a Cu Kα radiation (λ=1.54056nm) over a 2θ range of 3-60^0. The nature of chemical bonding of MMT and MMT-Citronella oil (10%) nanocomposite were determined by using Thermo scientific NICOLET Fourier transformation infra-red spectrometer in the range of 550-4000 cm^{-1}. Morphology and the surface topography of the clay composite can be obtained from Scanning electron microscopy (SEM). SEM images were obtained using a field emission scanning electron microscope (FE-SEM Hitachi SU6600).

Results and discussion

Powder X-ray Diffraction characterization (PXRD)

Referring to the first basal reflection (001) in PXRD, an interlayer spacing of 11.9 Å is seen for MMT and, with the introduction of Citronella oil (10%), the interlayer spacing of MMT increases to 12.6 Å (Figure 2). The increment between clay layers is an indication of the encapsulation of citronella oil (10%) into MMT.

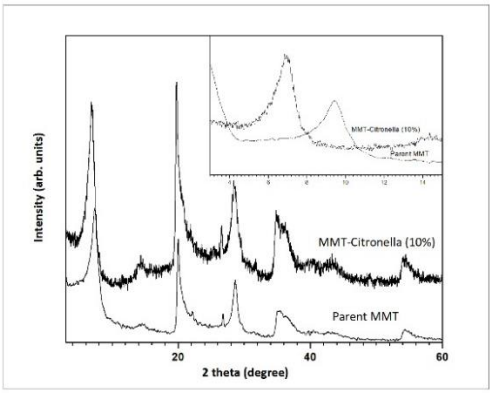


Figure 2. PXRD pattern of Na-MMT-Citronella oil (10%) nano clay composite

Fourier Transformation Infrared Spectrometry (FTIR)

Citronella showed a vibration peak of 2917 cm^{-1}, which express C-H bonding and it can be clearly identified in the spectrum of MMT-Citronella oil (10%) (Figure 3). This proves that citronella oil is encapsulated with MMT nanoclay.

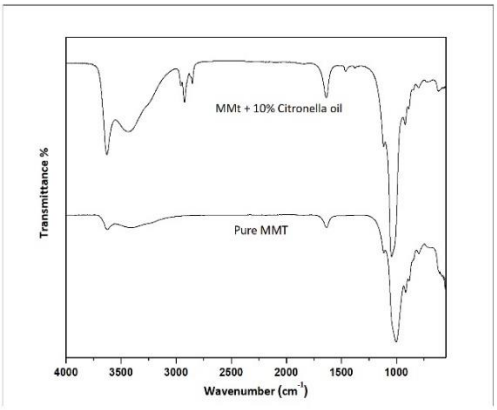


Figure 3. FTIR spectrum of Na-MMT-Citronella oil (10%) nanocomposite

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopic images indicated that even after grinding, typical plate like layered structure of clay remains unchanged (Figure 4).

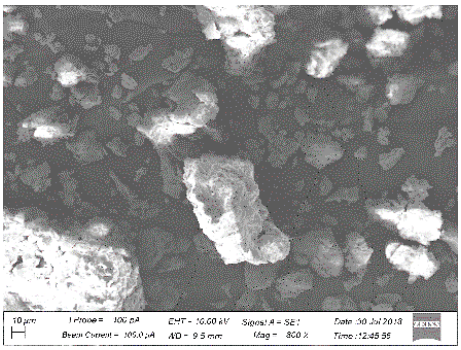


Figure 4. SEM image for Na-MMT-Citronella oil (10%) nanocomposite

Conclusions and recommendations

Citronella oil encapsulated MMT can be successfully prepared via liquid assisted grinding (LAG). Encapsulation of citronella oil into the interlayer space of MMT, provides a matrix to hold citronella oil molecules. This composite can be a promising material for mosquito repellence. The mosquito repellent behavior and its capacity to act as an air freshener are to be tested.

Mosquito repellency test will be done by using the cage test [5]. This method will be modified according to test the repellency of mosquitoes. Cage (30 cm × 15 cm × 15 cm) will be separated to two adjacent chambers, which mosquitoes can fly across. Adult female mosquitoes (ex: 10 mosquitos) will be put into the chambers separately. The clay composite will be place in one chamber and after 3 minutes count of the mosquitoes in each chamber will be taken. This will be repeated for several times and the percentage of mosquitoes in the chambers will be calculated.

References

[1] J. Islam, K. Zaman, S. Duarah, P. S. Raju, and P. Chattopadhyay, "Mosquito repellents: An insight into the chronological perspectives and novel discoveries," *Acta Trop.*, vol. 167, no. 2, pp. 216–230, 2017.

[2] E. Deletre *et al.*, "Prospects for repellent in pest control: current developments and future challenges," *Chemoecology*, vol. 26, no. 4, pp. 127–142, 2016.

[3] A. El Asbahani *et al.*, "Essential oils: From extraction to encapsulation," *Int. J. Pharm.*, vol. 483, no. 1–2, pp. 220–243, 2015.

[4] J. U. Rehman, A. Ali, and I. A. Khan, "Plant based products: Use and development as repellents against mosquitoes: A review," Fitoterapia, vol. 95, pp. 65–74, 2014.

[5] A. A. Anuar and N. Yusof, "Methods of imparting mosquito repellent agents and the assessing mosquito repellency on textile," *Fash. Text.*, vol. 3, no. 1, p. 12, 2016.

ECHOLOCATION CALL CHARACTERISTICS OF RHINOLOPHID BATS IN SRI LANKA

T.G.T. Kusuminda*[1], A. Mannakkara[1], C.J. Amarasinghe[2], K.A.P.M.K. Karunarathna[3], B.D. Patterson[4], W.B. Yapa[5]

[1]Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka
[2]Faculty of Graduate Studies, University of Colombo, Colombo, Sri Lanka,
[3]Sandaraja Wana Arana, Dothalu-Oya, Dolosbage, Sri Lanka
[4]Integrative Research Center, Field Museum of Natural History, Chicago, Illinois, USA
[5]Department of Zoology and Environment Sciences, Faculty of Science, University of Colombo, Sri Lanka
**Corresponding author (email: t.kusuminda@gmail.com)*

Introduction

The global success, species richness, and ability of bats to exploit diverse niches are mostly due to their capacity for powered flight and echolocation. Echolocation involves the production and emitting of ultrasonic sound waves and use the echoes they receive to gather information about their immediate environment. This acoustic process is mainly ultrasonic and allows bats to navigate complex three-dimensional spaces and find their insect prey in complete darkness. Echolocation involves strong selective pressure on signal design, favouring species-specific signal types linked to ecological conditions. Sri Lanka is a tropical island located near the Indian subcontinent and is known to support 30 species of bats representing 8 families. The family Rhinolophidae includes only two species in Sri Lanka, currently identified as *Rhinolophus rouxii* (Temminck) and *Rhinolophus beddomei* (Andersen). Basic information on the activity of different bat species, their distribution and habitat requirements can be gathered from their echolocation calls, if species specific echolocation calls can be recognised. Call recognition permits the development of comprehensive survey and monitoring methods essential for understanding the current status of bats and allow future monitoring of population parameters.

Traditional survey methods for bats involve capturing bats using mist nets and/or harp traps employed only a few metres above ground level. Therefore, even the most intensive studies typically fail to capture species that normally fly in open areas and above the forest canopy. Detecting bats from their calls is widely regarded as a means of overcoming these restrictions. Recent studies have shown that acoustic identification of Asian bat species is feasible and that acoustic methods are indispensable for maximising sample completeness in field surveys, an essential requirement for effective conservation planning [1, 2, and 3].

However, the use of ultrasound detectors in the tropics has been hindered by the lack of reliable call libraries, which allow identification of bats to genus or species level from their echolocation calls. For this reason, acoustic sampling has been rarely employed in the region and detailed descriptions are lacking for the echolocation calls of most bat species in Sri Lanka. We address this by providing the first detailed description and comparison of time-expanded echolocation calls of the two bat species in the genus *Rhinolophus* in Sri Lanka and elsewhere in their geographic range.

Materials and Methods

Study site

Rhinolophus rouxii was captured at six locations in Sri Lanka including Thawalama (6.36958N and 80.32442E) in low country wet zone, Idulgashenna (6.77678N and 80.89058E) in up country intermediate zone, Udupussellawa (6.96892N and 80.90142E) in up country intermediate zone, Radella (6.92983N and 80.72708E) in up country wet zone, Yatideriya (7.12794N and 80.36831E) in mid country wet zone and Ridigama (7.53731N and 80.49206E) in low country intermediate zone. *Rhinolophus beddomei* was captured at two locations including Deraniyagala (6.94981N and 80.33344E) in low country wet zone and Thawalama where the *R. rouxii* was sampled.

Capture methods and species identifi cation

Bats were captured at their roosting sites and inside tea plantations during ongoing research using mist nets, a harp trap, and on certain occasions by a hand net. During mist netting, a single 2.5x12 m mist net (mesh size 38 mm) was set in the evening at ground level and kept open until the following morning. A two-bank harp trap with capture area of 1.8x2.4 m was placed in front of selected roosting sites. All captured bats were immediately transferred to cotton bags to reduce their stress. After that, key external morphometric measurements were taken using digital calipers. Species identification was confirmed using a regional bat key [4].

Acoustic methods and call measurement

Echolocation calls of bats were recorded as sound files (.wav) from motionless bats held in the hand using a Pettersson M500 microphone (Pettersson Elektronik AB, Sweden) and BatSound Touch software (Pettersson Elektronik AB, Sweden) on an ASUS Core i5 laptop (ASUSTek Computers Inc., Taiwan). A total of 82 unambiguous echolocation calls with high signal-to-noise ratio, emitted by 19 (*R. rouxii*) and 4 (*R. beddomei*) individual bats were selected for further analyses. Bat echolocation calls were analysed using BatSound Pro v.3.32 (Pettersson

Elektronik AB, Sweden) with software settings of 1024-size Fast Fourier Transformation (FFT), with 95% overlap in a Hanning Window. Frequency of maximum Energy (FME) or Peak Frequency was measured using Power Spectrum. Call duration (CD) was measured in milliseconds (ms) from the Oscillograms [1]

Statistical procedures

To test the efficacy of acoustic data in correctly identifying bat species, analysis of variance (ANOVA) was conducted to examine significant differences in the multiple variables. Levene's test for homogeneity of variance was used before the comparison of means to assess the equality of variances. All tests were performed using Minitab (vers. 17.0). In all tests, values of $P < 0.05$ were considered as significant.

Results and Discussion

The two *Rhinolophus* species produced calls characterised by a long constant frequency (CF) component which was preceded and terminated by a brief frequency-modulated (FM) component. FME (frequency of maximum energy) values (mean ± SD) were 74.0 ± 1.1 kHz in *R. rouxii* and 52.7 ± 0.2 kHz in *R. beddomei*, whereas call duration was 54.9 ± 6.9 ms in *R. rouxii* and 56.2 ± 3.2 ms in *R. beddomei*. FME values were not overlapping between species, indicating this call parameter permits the field identification of rhinolophid species in Sri Lanka.

According to the results of ANOVA, these bat species could be significantly differentiated by FME. The analysis of variance results for FME was highly significant ($F = 15745.03$, $P = 0.000$).

Ours is the first study to characterise the echolocation calls produced by Sri Lankan rhinolophid bats. Our results indicate that correct acoustic identification of in-country bat species is feasible using the call parameters (especially FME) we employed. Interestingly, each of the *Rhinolophus* species recorded in the present study varies in call frequency to some degree across their biogeographic ranges (Table 1). Biogeographic variation in call frequency may be due to the existence of cryptic species or subspecies or related to ecological constrained imposed by the different habitat structures. Bats that emit CF calls at different frequencies often prove to be different cryptic species [5]. Recently, two lineages of *R. rouxii* inhabiting South India with modestly different calls were found to be divergent genetically and were separated in to two species as *R. rouxii* and *R. indorouxii* [4]. The highly distinctive call differences between both Sri Lankan species and their supposed conspecifics in India raises questions concerning their identity and taxonomy. Detailed molecular and morphometric studies combined with acoustic

information on Sri Lankan species are warranted to inventories Sri Lanka bat species.

Table1. Comparisons of echolocation call parameters of *R. rouxii* and *R. beddomei* in its geographical range. Data are given as mean ± SD (min-max)

Data source; country; number of individual bats (*n*)	Call characters (*R. rouxii*)		Data source; country; number of calls (*n*)	Call characters (*R. beddomei*)	
	FME (kHz)	CD (ms)		FME (kHz)	CD (ms)
This study; Sri Lanka; *n=19*	**74.0 ± 1.1** (71.5-77.1)	54.9 ± 6.9 (43.0-70.9)	This study; Sri Lanka; *n=4*	**52.7 ± 0.2** (52.6-53.4)	56.2 ± 3.3 (48.1-64.0)
[4]; Moodbidri, India; *n=32*	**80.9 ± 1.1** (77.9–83.3)	46.1 ± 6.3 (31.3–60.6)	[4]; Tamil Nadu, India	**42.81 ± 0.5** (41.7–43.3)	47.7 ± 13.6 (24.7–71.3)
[4]; Mahabaleshwar, India; *n=6*	**82.8 ± 1.3**	50.0	[4]; Thailand; *n=1*	**49.3**	36-39

Conclusions and Recommendations

Acoustic identification of bats is perhaps best considered as a valuable addition to traditional capture methods, rather than as a replacement for them. As the taxonomy of many Asian bats remains uncertain, the echolocation calls and genetic divergence of Sri Lankan bat species must be compared to those from the mainland to assure proper nomenclature. We strongly recommend further studies to facilitate the development of echolocation call libraries as a tool for improving identification and conservation of Sri Lankan bats.

Acknowledgements

Our sincere gratitude extends to the owners and managers of all sampling sites and their staff who provided their support. We would like to thank staff members of Tea Research Institute at Talawakelle (especially Rajika Gamge), Hanthana, Ratnapura and Passara for help in finding suitable sampling sites. Sameera Karunarathna, Krishan Wewalwala, Srinath Amarasinghe, Nilupul Rangana, Malinga Pathum, Dilshan Madusanka, Kavishka Chathuranga and Asanka

Tennakoon are thanked for various sorts of support. Further we would like to thank the Department of Wildlife Conservation for issuing the research permit (WL/3/2/02/2016) and Institute of Biology for issuing the ethical clearance certificate. Financial assistance was provided by the National Research Council; under Grant 15-111, and Rufford Foundation under Grant 17065-1.

References

[1] A.C. Hughes, C. Satasook, P.J.J. Bates, P. Soisook, T. Sritongchuay, G. Jones and S. Bumrungsri. "Using echolocation calls to identify Thai bat species: Vespertilionidae, Emballonuridae, Nycteridae and Megadermatidae." *Acta Chiropterologica*, vol. 13, pp. 447-455, Dec. 2011.

[2] P. Sophany, P. Sarith and N.M. Furey. "Cambodian bat echolocation: a first description of assemblage call parameters and assessment of their utility for species identification." *Cambodian Journal of Natural History*, vol. 2013, pp. 16-26. Aug. 2013.

[3] C.F.R. Wordley, E.K. Foui, D. Mudappa, M. Sankaran and J.D. Altringham. "Acoustic identification of bats in the Southern Western Ghats, India." *Acta Chiropterologica*, vol. 16, pp. 213-222, Jun. 2014.

[4] C. Srinivasulu, P.A. Racey and S. Mistry. "A key to the bats (Mammalia: Chiroptera) of South Asia." *Journal of Threatened Taxa*, vol. 2, pp. 1001-1076, Jul. 2010.

[5] T. Kingston, M.C. Lara, G. Jones, Z. Akbar, T.H. Kunz and C.J. Schneider. "Acoustic divergence in two cryptic *Hipposideros* species: a role for social selection?" *Proceedings of the Royal Society B: Biological Sciences*, vol. 268, pp. 1381–1386. Jul. 2001.

ASSESSING PADDY FARMERS' WILLINGNESS-TO-PAY TOWARDS ECO-FRIENDLY TECHNOLOGIES AIMING THE REDUCTION OF CHEMICAL FERTILIZER USAGE

L.H.N. De Silva*[1], C.D.A. Lakmali[1], U.K. Jayasinghe-Mudalige[1], R.S. Dharmakeerthi[2], W.S. Dandeniya[2], W.K. Balasooriya[3]

[1]Department of Agribusiness Management, [3]Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP)
[2]Department of Soil Science, Faculty of Agriculture, University of Peradeniya, Peradeniya
**Corresponding author (email: nadeera.de@hotmail.com)*

Introduction

Green revolution enhances the food production by using high yield varieties and Chemical Fertilizer (CF). Excessive use of CF leads to increased environmental pollution, negatively affect human and ecosystem health, social and political unrests in the country. Development of Eco-Friendly Technologies (EFTs) is, therefore, become a necessity to reduce the usage of CF, while maintaining higher productivity and improving soil fertility.

Agriculture remains as a key sector for the economic development in Sri Lanka, while rice production is considered to be the primary form of it. Incorporation of organic manure with CF is highly recommended for a higher yield since majority of soils in Sri Lanka are very poor in soil fertility. Even though rice cultivation based on the organic fertilizer, easy handling and heavily subsidized schemes leads to trigger the excessive use of CF [1]. Rice considered to be the crop which utilizes nearly 70% of the annual inorganic fertilizer imports. Additionally, 50-70% of CF used in paddy cultivation loss due to several processes that leads to the low fertilizer use efficiency. Under these circumstances, a proper mechanism is required to reduce environmental damage caused through CF.

In this multi-year multi-objective research study, "Bio-fertilizer" (EFT_1) and "Slow releasing urea using rice husk bio-char" (EFT_2) are in the process of introducing as EFTs with the aim of increasing the fertilizer use efficiency and reducing the frequency of CF application. EFT_1 was formulated using microbial inoculants that improve nutrient availability to plants. The farmers may expect that inoculant to be compatible with routine field practices and ease of use, etc. Therefore it is important to introduce bio-fertilizers that could be applied to rice-based cropping systems to reduce the CF usage while improving soil health. EFT_2 considered being important when reducing the Nitrogen losses in the field. Highly porous structure and superior properties of bio-char [2] was considered when coating urea granules with rice husk bio-char to produce it cost effectively. EFT_2 will not only reduce chemical fertilizer usage in the country, but also the application of bio-char into the soil every season could contribute to improve soil fertility gradually.

It is important to look into socio-economic aspects of production of a new technology since "profitability" is matter for farmers to adopt any technology in their field. Farmer's decision to adopt an innovative technology depends highly on profits (increased benefits) over the expenses (decreased costs). What factors trigger farmers to adopt EFTs in the field, and more importantly, the "role of economics" in adoption of such technologies is, however, not yet fully disclosed. Having recognized the most important attributes pertaining to the benefits and costs, the purpose of this analysis is to find the extent to which those potential end-users in the paddy sector are willing to pay for the deliverables of ETFs, individually and collectively. The appropriate 'Choice Modelling' techniques, as applicable to the real case in practice, was adopted to estimate the WTP of those potential 'end-users'. The idea is to see the extent to which the 'potential followers' are willing-to-pay for an innovative package of such technologies that replace the CF. It helps to get clear knowledge of which attributes are most important when selecting a choice. The study focused on investigating what farmers considered when using the proposed EFTs.

Methodology

Theoretical framework

Choice Experiment (CE) Technique was used to determine the values of individual attributes and technologies. It is based on the assumption of the utility-maximizing behavior of the respondent. CE was carried out based on two fundamentals, including Lancaster's Characteristics Theory [3] and Random Utility Theory.

Marginal welfare measure that seeks for a change in any of the attributes known as Marginal Willingness-To-Pay (MWTP), which is calculated using the ratio of the coefficients (β) of the respective attribute and the monetary attribute, holding all else equal. MWTP calculated as:

$$\mathrm{MWTP}_{\mathrm{attribute}} = -1\left(\frac{\beta_{\mathrm{attribute}}}{\beta_{\mathrm{monetary\ attribute}}}\right)$$

Choice sets

Choice cards for two different EFTs were prepared separately using crucial attributes and attribute levels which selected through a series of focus group discussions and past literature [4] (Table 1). Under the full factorial design, 64 possible choice alternatives were prepared using six attributes and two attribute level per each.

Orthogonalization procedure was completed to reduce the choices up to sixteen possible choice combinations and randomly blocked into eight different versions including two options per each technique.

Table 1. Selected attributes and attribute levels for the proposed technologies

Bio-Fertilizer		Slow Release Urea using Rice-husk bio char	
Attributes	**Levels**	**Attributes**	**Levels**
Preferred Form	Liquid Form* Powder Form	**Environmental Damage**	High* Low
Nutrient Solubilizing Rate	Low * High	**Availability to Farmers**	Farmer Societies* Private Markets
Purchasing from	Through RASS* Through Producer Outlets	**Yield Increment**	Same* High
Promotional Activities	Field Demonstrations* Farmer meetings	**Soil Fertility Improvement**	Low* High
Environmental Damage	High* Low	**Integration with Other Technologies**	Difficult to integrate* Easy to integrate
Cost of Fertilizer Application (per acre)	25% lower than current practice 40% lower than current practice	**Cost of Urea Application (per acre)**	25% lower than current practice 30% lower than current practice

**Reference level, RASS – Regional Agricultural Service Stations*

Collection and analysis of data

Those paddy farmers belong to Pannala area located in the Kurunegala district were selected randomly (n=40) and a structured questionnaire-based face-to-face interviews were conducted to collect data. Farmers were presented with a "Choice Card" (i.e. 1 out of 8 prepared with varied combinations) and asked to select the 'best-preferred option'.

Conditional Logistic Regression (CLR) was employed to assess the values of EFTs. Two separate CLR analysis were conducted for EFT_1 and EFT_2. Dummy coding has been applied for the attribute levels and level with least preference indicated as the reference level. Remaining levels were estimated by setting reference level to zero.

Results and Discussions

Descriptive statistics of the sample claims that, majority of the respondents (73%) were male and almost 45% of the respondent educated up to ordinary level. Further, results indicate that almost 55% do have an extra income apart from paddy farming. Moreover, paddy farming land area of respondents ranged from 0.5 to 5 Acres and nearly 85% of the respondents own their land and rest does farming on rent basis (*ande*). Unavailability of an efficient workforce and high cost leads to perform broadcasting technique for seeding procedure. Survey statistics show that almost 75% of farmers prefer material based fertilizer subsidy scheme.

Almost more than 80% of the sample were unaware of the terminologies of bio fertilizer and bio char.

With reference to choice experiment results, parameters of the utility equation illustrate how attributes are relatively important and influence on individual preference. It is difficult to draw direct conclusions on the parameter size while using CLR. But the sign of an individual parameter indicates the positive or negative impact of an attribute on the total utility of respondents. The results obtained from CLR illustrates that availability of EFT_1 in powder form and high land productivity respective to use of EFT_2 were significant at 95% of significant level. Apart from that low environmental damage was significant with respect to both EFTs. Significant attributes were assumed to be relevant when respondents selecting a choice.

Coefficients of the cost of fertilizer indicated to be negative and highly significant in both technologies. Thus, the sign indicates that the utility of choosing a choice set is negative with a higher cost. Further, negative sign (i.e. EFT_1) for the attributes of conducting promotional activities through farmer meetings reinforce that our respondents prefer field demonstrations to learn about innovative techniques.

Available results from CLR model used to calculate MWTP, where it indicates how much farmers are willing to pay to enhance the attribute. The analysis provides a better understanding of the relative importance of the attribute to farmers where potential investors are benefited through it. According to the MWTP values obtained respective to EFT_1, paddy farmers were willing to pay the highest value of Rs. 270.00 for low environmental damage while they willing to pay Rs. 153.00 for powdered form of fertilizer and Rs. 117.00 for fertilizer availability through producer outlets respectively. They claimed that inefficiency and unavailability of appropriate fertilizer amounts in government oriented societies lead to the dissatisfaction towards those practices. Farmers prefer better, efficient performance while incorporating private parties in the process. However, Rs. 189.00 will be required to compensate farmers to undergo training and promotional activities done through the farmer meetings.

With respective to MWTP analysis on EFT_2 surveyed farmers were willing to pay for all attributes considered except availability to the farmers through private markets. Farmers ranked the highest MWTP value of Rs. 81.00 for low environmental damage. Apart from that they willing to pay an extra amount of Rs. 60.00 for higher land productivity, Rs. 12.30 for both high soil fertility improvement and easy integration ability with other technologies. On the other hand negative value for MWTP implies that farmers were not willing to pay extra to purchase newly introduced slow release urea fertilizer from an external private market since farmers highly rely on the commercially available urea fertilizer.

Conclusions and Recommendations

Introduction of this kind of EFTs can significantly benefit on Sri Lanka's economy. The outcome of the analysis elicits that potential end users widely preferred for the environmental benefits gained through new technologies. It was reported that highest demand was given to low environmental damage with regarding to newly developed EFTs. Socio-economic analysis implicit that farmers preferred to have eco-friendly technologies, which are easily manageable to whole farmers. Most importantly, the technologies developed for the purpose should be easily transferable to farmers without affecting their income level.

Since farmers highly preferred fertilizer subsidy scheme, it would be much profitable if these technologies to be incorporated with a respective scheme. Presenting of high-quality product with a good assurance of performance leads to attract farmers. Even though there is a negative impact on government based actions, positive impression on EFTs and properly managed fertilizer production, distribution and promotion activities would lead to enhance the use of EFTs. It is better to undergo a detailed financial analysis to further interpret on business perspective.

Acknowledgement

Financial assistance by National Research Council of Sri Lanka under the Research Grant: TO 16 - 07.

References

[1] H. Herath, E. Gunawardena, and W. Wickramasinghe, "The impact of 'Kethata Aruna' fertilizer subsidy programme on fertilizer use and paddy production in Sri Lanka," *Tropical Agricultural Research*, vol. 25, no. 1, p. 14, 2015.

[2] D. Filiberto and J. Gaunt, "Practicality of Biochar Additions to Enhance Soil and Crop Productivity," *Agriculture*, vol. 3, no. 4, pp. 715–725, 2013.

[3] K. J. Lancaster, "A New Approach to Consumer Theory," *Journal of Political Economy*, vol. 74, no. 2, pp. 132–157, 1966.

[4] J. Tur-Cardona, O. Bonnichsen, S. Speelman, A. Verspecht, L. Carpentier, L. Debruyne, F. Marchand, B. H. Jacobsen, and J. Buysse, "Farmers reasons to accept bio-based fertilizers: A choice experiment in seven different European countries," *Journal of Cleaner Production*, vol. 197, pp. 406–416, 2018.

REAL-TIME IMAGE SURVEILLANCE OVER IOT FOR INTRUSION DETECTION

Y. S. P. Weerasinghe*[1], P. Saengudomlert[2]

[1]*National Engineering Research and Development Center Sri Lanka.*
[2] *Bangkok University Center of Research in Optoelectronics, Communications and Computational Systems*
**Corresponding author (email: praveen@nerdc.lk)*

Introduction

Internet of Things (IOT) is widely used in many applications which connect devices in places over the Internet [1]. IoT plays the main role in current home automation systems. Intrusion detection, surveillance cameras, controlling of domestic appliances, and monitoring of energy consumption are some applications that can be controlled remotely over the Internet. With intrusion detection, when a home resident is out, he can know what happens inside the home, especially when an unauthorized person enters.

An effective and affordable home security system should be cost-effective and able to facilitate real-time access [2]. Most intrusion detection systems were based on controllers such as Arduino, Raspberry PI, ESP 8266, and wireless technologies such as Wi-Fi and ZigBee, as well as their combinations with remote access in real time [3], [4], [5]. The most widely used motion detection sensor is a passive infrared (PIR) motion sensor which is considered robust in most types of environments [5]. In addition, a magnetic reed contact sensor is widely used to detect whether a door is open or closed. These sensors can interface with different communication technologies of GSM and 3G with remote server and remote access facilities [3], [4]. However, with the use of multiple controllers, the system might be complex, inefficient, and expensive.

The main objective of this research is to develop a cost-effective intrusion detection system with real-time access and remote alert features. The intrusion detection system should be simple and small so that the user can easily adopt. For hardware, sensor devices include a PIR motion sensor, a magnetic reed contact sensor, and an Arducam Mini camera. They are connected to an ESP-12E microcontroller with a Wi-Fi access. The ESP-12E connects to a remote server, which allows a registered remote user to check the current status of the system via a web interface. Upon detecting an intrusion, an alert message is sent to the remote server using the IoT platform. The message reaches the user as a notification on the user's smart phone.

Materials and Methods

Figure 1 shows the proposed system architecture for an IoT based intrusion detection system. PIR and magnetic reed sensors are connected to the ESP-12E

microcontroller, which is also connected to the Arducam Mini camera module with the image resolution of 2 megapixels. The ESP-12E microcontroller operates as a master controller and a server over the IEEE 802.11 interface, which updates the sensor states and the captured images. The server connects to the remote server in the IoT platform via the Internet. The Blynk server is used as the remoter server. An Internet connection with a static IP address is used to access the server remotely.

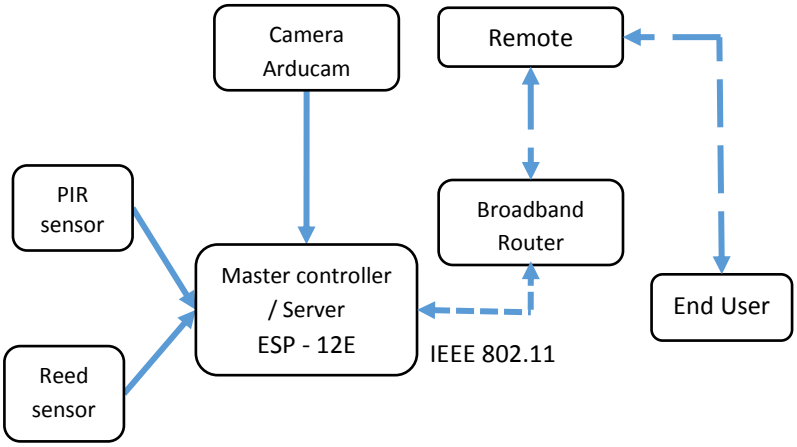


Figure 1. Basic system architecture

When the PIR sensor or the reed sensor detects an intrusion, it triggers the microcontroller to intiate the Arducam capturing mode. The captured images are stored in the server flash memory. The user can connect to the server using the static IP address and access the HTTP server in ESP-12E. The server displays the states of the PIR motion sensor and the reed sensor. It also stores the captured images with the resolution of 1280×1024. The Arducam capturing resolution can be adjusted from 160×120 to 1600×1200. The server is refreshed every 10 seconds in order to provide the web page with the latest updates. Upon detecting an intrusion, a notification alert is sent to the Blynk server, which alerts the user's smart phone. The user needs to install the Blynk application and register to get such notifications. When a notification alert reaches the user's smart phone, the user can access the HTTP server over the Internet in order to see what has happened at home. Therefore, the system will keep the user informed about each possible home intrusion.

Results and Discussion

The screenshot in figure 2 shows how the HTTP server displays the notifications and the stored images in the JPEG format. The server memory can be cleared when the buffer storage is full. The captured images can get distorted when the file space is full, and it is an option that the user can delete the old images in the server memory. The average image capturing time is 21 ms while the image

saving time is 1,691 ms. The captured images are continuously updated on the HTTP server in real time.

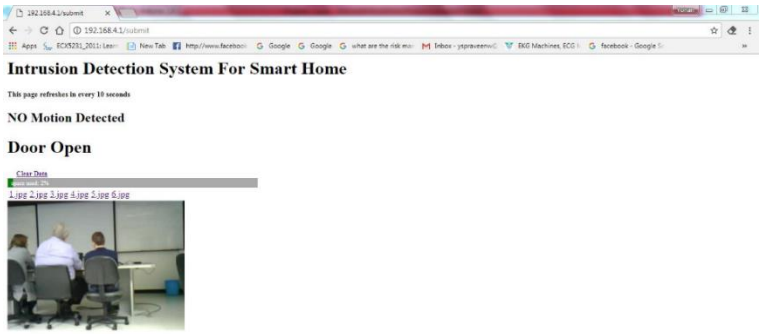


Figure 2. Example webpage access edby the user

The intrusion detection system has several advantages over the existing home security systems. It is small in size, and can be placed anywhere without any dedicated installation platform and wiring. The system was developed using the ESP-12E module and integrated sensor modules that are cost-effective. Over the Internet, the user can monitor the images which are stored in the server, in addition to the current status of the PIR sensor and the reed sensor. The user can access images stored in the past to learn about past activities. The special feature of an offline mode notification alerts the user when the system is about to stop functioning because of a power failure or a system malfunction; this was not available in existing home security systems. The server also has a customized interface that the user can use to adjust the resolution, an alert notification method, and so on.

The features of the developed system were not available as a whole in other intrusion detection systems. The developed intrusion detection system has the features of detecting an intruder's arrival and capturing images. The captured images are stored in the memory module in the camera and can be accessed remotely by using a static Internet Protocol (IP) address. As a whole the system can be developed with the initial cost of 13000 LKR. The system can be developed by local small-scale entrepreneurs and can be used as an ideal device in smart home applications.

Apart from the advantages, there are some disadvantages. The system needs to connect to the Internet using a home broadband router with NAT configuration. The user also needs to purchase a static IP address to connect remotely. In the existing systems, this facility is provided by cloud based systems. The user logs into the remote server and access the real-time images captured in the home environment. The point-to-point connection between the user and the HTTP server will be affected by the bandwidth of the medium, which will in turn affect

the real-time status update and the image quality. The ESP-12E microcontroller and the integrated modules operates based on a 3.3V DC supply, and hence needs a special power supply to be used in home environments. Finally, some commercial systems available in the market have autorotation of images and night-vision facilities in the camera, which are not provided in our system.

Conclusions and Recommendations

This work implements a cost-effective intrusion detection system which can monitor, capture and store images in a server supporting a remote access. Image capturing by the the Arducam Mini module in an event of an intrusion is triggered by either the PIR sensor or the reed sensor. The intrusion detected by either sensor is reported to the user's smart phone in the form of an alert message. Then, the user can access the captured images that are stored in the HTTP server, which utilizes the ESP-12E microcontroller memory. The server can be accessed by the user remotely over the Internet using a public IP address.

The current system captures images continuously whenever the PIR or the reed sensor detects an intrusion. These images can fill up the microcontroller memory. One future work is to modify the system to automatically store only the most recent images in the server memory. In addition, a timestamp should be printed on each stored image and the state changes of the sensors that trigger alert messages. Accordingly, the user can find out the time of each intrusion event that happened inside his home.

References

[1] Kodali R.K., Jain V., Bose S., Boppana L., IoT based smart security and home automation system, in International Conference on Computing, Communication and Automation (ICCCA), 29-30 April 2016 (pp. 1286-1289).

[2] Kishore P., Veeramanikandasamy T., Sambath K., and Veerakumar S., Internet of Things based low-cost real-time home automation and smart security System, International Journal of Advanced Research in Computer and Communication Engineering (IJARCCE), vol. 6, no. 4, April 2017 (pp. 505-509).

[3] Prasad S., Mahalakshmi P., Sunder A.J., Swathi R., Smart surveillance monitoring system using Raspberry Pi and PIR sensor, International Journal of Computer Science and Information Technologies (IJCSIT), vol. 5, no. 6, June 2014 (pp. 7107-7109).

[4] Chandramohan J., Nagarajan R., Satheeshkumar K., Ajithkumar N., Gopinath P.A., Ranjithkumar S., Intelligent smart home automation and security system using Arduino and Wi-Fi, International Journal of Engineering and Computer Science (IJECS), vol. 6, no. 3, March 2017.

[5] Sahoo K.C., Pati U.C., IoT based intrusion detection system using PIR sensor, Recent Trends in Electronics, Information & Communication Technology (RTEICT), 19 May 2017 (pp. 1641-1645).

REMOVAL OF METHYLENE BLUE ADSORPTION ONTO ACTIVATED CARBON DEVELOPED FROM CINNAMON WOOD

N.W.P.S. Perera[1]*, C.A.N. Fernando[1], S.A. Ariadurai[2]

[1]Nano-Technology Research Laboratory, Department of Nano sceinces & Faculty of Technology, Wayamba University of Sri Lanka, Kuliyapitiya
[2]Department of Textile & Apparel Technology, Faculty of Engineering Technology, Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka
**Corresponding author (email: pradeep@wyb.ac.lk)*

Introduction

Activated carbons are prepared by different methods and from various raw materials. However, commercially available activated carbon is expensive. In recent times, much research work has been focusing on finding low-cost precursors and methods [1] to produce activated carbon.

Activated carbon has many applications [2]. One of which is adsorbent agent for purification of liquid and gas phase applications. Activated carbon has been an effective adsorbent for MB dye removal in industrial effluents. Adsorption capacity of carbon is known to be dependent on the porous structure and surface charges. Surface charges are mainly created by nature of the functional groups [3].

Cinnamon wood is a hard biomass that is separated from cinnamon bark during the manufacturing of cinnamon. It's porous nature and higher amount of lignin and cellulose content is suitable for activated carbon production. Present work investigates the removal of MB from aqueous medium using activated carbon prepared from CWAC

Materials and Methods

Adsorbent

Two year's old cinnamon tree stem wood was collected from the southern province of Sri Lanka. Firstly, the wood was cut into small pieces and dried in the sunlight. After this, cinnamon pieces are bio scoured and dried in the muffle oven at 105 ^{0}C for 4 hours. Activated carbon was prepared by chemical activation by treating with 0.1 M of Potassium Hydroxide (KOH) of raw cinnamon wood particles at 410 ^{0}C at a heating rate of 10 ^{0}C min^{-1} in nitrogen atmosphere for 0.15 h. After cooling particles to room temperature, the resulting activated carbon (CWAC) was preserved and used as an adsorbent. CWAC was characterized by using proximate analysis, SEM, and EDS techniques. [4]

Adsorbate

Methylene blue solution was prepared by dissolving the required amount of dye in distilled water.

Experiment protocol

The batch type adsorption experiments were conducted in a set of 100 ml of beaker containing adsorbent and 50 ml of MB solution with different concentrations. The beaker was agitated at 600 rpm at 27 ± 1 ^{0}C until the equilibrium level. After centrifuging at 6000 rpm for 7 min, the final concentration of dye in the centrifuged solution was measured at 665nm using the UV-Visible spectrophotometer. The pH of the solution was adjusted with 1N HCl and 1N NaOH solutions. The amount of dye adsorbed and percentage removal of MB was calculated using the following expression.

$$\% \text{ Removal} = \frac{(C_i - C_e)}{C_i}$$

$$q_e = (C_o - C_e)\frac{V}{M}$$

q_e amount of dye in mg per gram of adsorbent

C_i, C_o initial concentration

C_e Equilibrium concentration

V Volume of solution

M mass of adsorbent

Adsorption isotherm

Langmuir isotherm

Langmuir isotherm model proposed by Langmuir in 1916 is applied for equilibrium sorption assuming monolayer coverage on a surface [5].

$$\frac{C_e}{q_e} = \frac{1}{bq_m} + \frac{C_e}{q_m}$$

Where q_e is the amount of adsorbate adsorbed on the adsorbent (mg/g) at equilibrium, C_e is the equilibrium concentration (mg/L) of the adsorbate, q_m is the monolayer adsorption capacity (mg/g) and b is the Langmuir constant (L/mg) associated to the free energy of adsorption.

Results and Discussion

Characterization of CWAC

Proximate analysis results demonstrate those physical characteristics of the carbon prepared at 420 ^{0}C - moisture content (12 %), ash content (3.2 %), volatile content (35 %), and density (0.36 g/l).

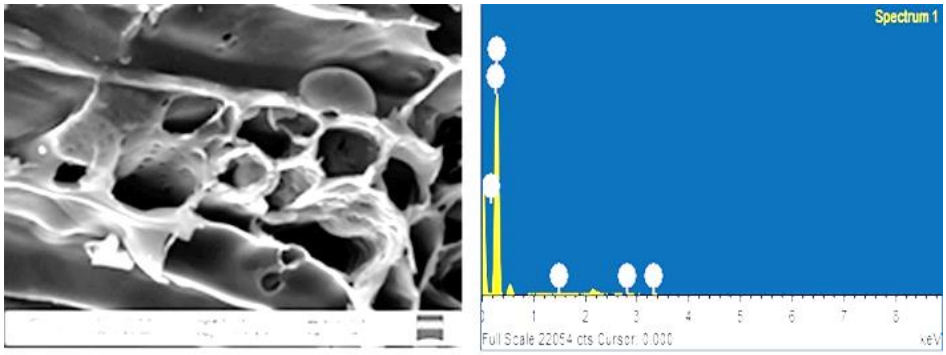


Figure 2. (a) SEM micrograph of CWAC (b) EDX Spectra of CWAC

Figure 1a shown the SEM micrograph of CWAC. According to this micrograph, uneven porous nature on the surface structure of CWAC couid be seen. Pore structure arrangement was seen as a honeycomb. This pore arrangement remarkably enhances the MB adsorption capacity. Elementary analysis results were shown in figure 1b. It indicates elements Carbon (97.68 %), chlorine (0.91%), and potassium (1.4%) in cinnamon activated carbon

Removal of MB

Effect of adsorbent dose

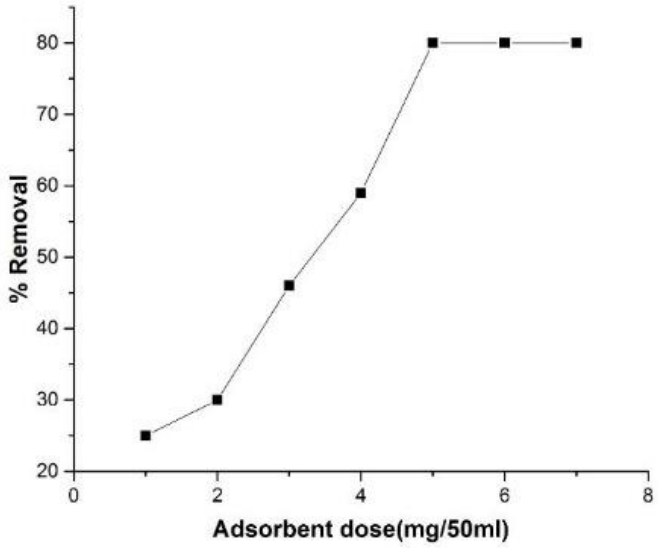


Figure 3. Effect of adsorbent on MB removal

The adsorbent mass changed from 0.1 to 1g/50 ml. This was shown in figure 2. MB removal increased gradually up to 0.5/50 ml. This may due to the number of pores and pore sizes. However, after 0.5g/50 ml, all adsorption sites were filled and saturation level has been reached. There is no increase in an effective surface area of CWAC.

Effect of contact time

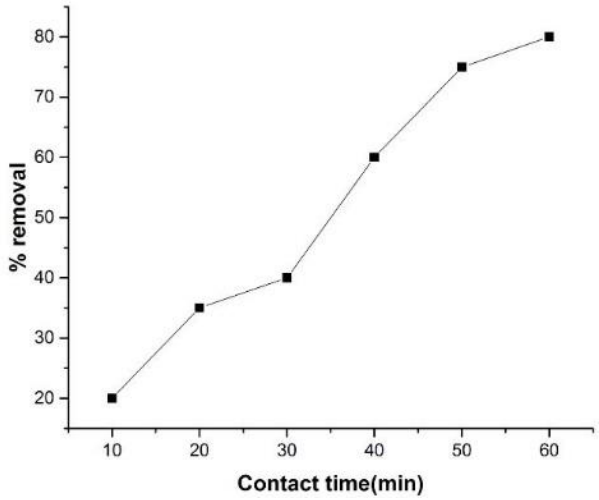


Figure 4. Effect of adsorbent on MB removal

The effect of contact time on removal of MB was shown in Figure 3. It could be seen 80% dye removal took place in 60 min for CWAC. In the initial stage, more adsorption side were available in CWAC and dye solution was highly concentrated. Later, the lower adsorption rate was due to a decrease in the number of vacant sides of CWAC and MB concentration. This shown a formation of the monolayer on the CWAC surface.

Effect of pH

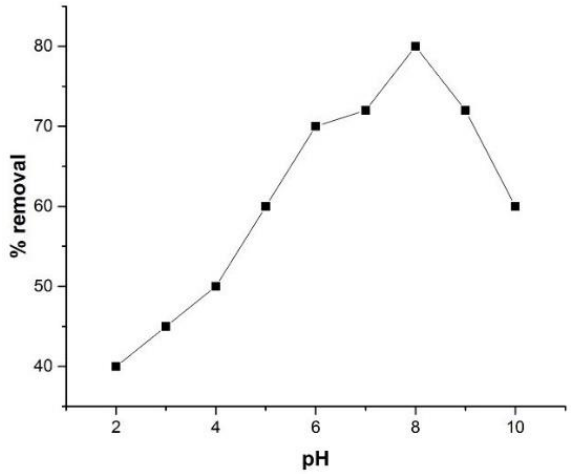


Figure 5. Effect of pH on MB removal

The effect of solute pH on the adsorption of dye was shown in Figure 4 for cinnamon activated carbon. The amount of adsorption increases when the pH increases. Higher pH was found to be favourable for maximum removal efficiency and was consistent with the results obtained for the surface charged measurement. The negatively charged carbon surfaces functional groups at pH greater than 3.6 could electrostatically interact with methylene blue cations.

Adsorption Isotherm

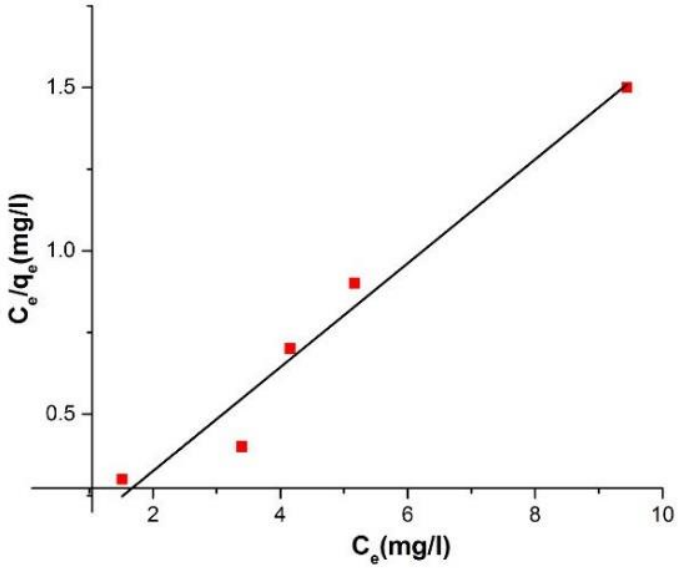


Figure 6. Langmuir plot for the adsorption of MB dye on Cinamon activated carbon.

Adsorption capacity and other parameters were determined using Langmuir model. Figure 5 represents the Langmuir model. It has been observed that the adsorption capacity is 72.2 mg/g. The high value of correlation coefficient (0.9655) indicates the applicability of Langmuir model, which reveals a monolayer coverage and formation of the uniform layer.

Conclusions and Recommendations

In this work, CWAC produced from cinnamon woood for first time and CWAC shows best adsorption capacity for MB removal. The operating parameters for the maximum sorption dose, contact time, and pH level have also been determined. Equilibrium data were fitted in the Langmuir model. This cinnamon wood, which is regarded as a waste and pollutant can be converted to a value-added product, which has extensive application for removing dye from water produced by various factories.

Reference

[6] Kan Y, Yue Q, Li D, Wu Y, Gao B. Preparation and characterization of activated carbons from waste tea by H3PO4 activation in different atmospheres for oxytetracycline removal. Journal of the Taiwan Institute of Chemical Engineers. 2017 Feb 1; 71:494-500.

[7] Taghipour T, Karimipour G, Ghaedi M, Asfaram A. Mild synthesis of a Zn (II) metal organic polymer and its hybrid with activated carbon: Application as antibacterial agent and in water treatment by using sonochemistry: Optimization, kinetic and isotherm study. Ultrasonics sonochemistry. 2018 Mar 1;41:389-96.

[8] Saleh TA, Sarı A, Tuzen M. Optimization of parameters with experimental design for the adsorption of mercury using polyethylenimine modified-activated carbon. Journal of Environmental Chemical Engineering. 2017 Feb 28;5(1):1079-88.

[9] Kazak O, E Kazak O, Eker YR, Bingol H, Tor A. Preparation of activated carbon from molasses-to-ethanol process waste vinasse and its performance as adsorbent material. Bioresource technology. 2017 Oct 1;241:1077-83.ker YR, Bingol H, Tor A. Preparation of activated carbon from molasses-to-ethanol process waste vinasse and its performance as adsorbent material. Bioresource technology. 2017 Oct 1;241:1077-83.

[10] Langmuir I. The constitution and fundamental properties of solids and liquids. Part I. Solids. Journal of the American chemical society. 1916 Nov;38(11):2221-95.

PARALLEL COMPUTING USING RASPBERRY PI: AN APPROACHABLE WAY FOR ENVIRONMENTAL MODELLING

G.R.L. Kodikara*

Department of Geosciences, University of Wisconsin-Milwaukee, USA
**Corresponding author (email: gayantha@uwm.edu)*

Introduction

Integrated spatial-temporal modeling of complex environmental systems typically requires high resolution of spatial and temporal data. Most of the natural systems are also heterogeneous over the landscape and time [1]. Moreover, these systems are generally consisting of complex nonlinear interactions between different system features, creating uncertainty and sensitivity issues of the model when it represents the natural processes [1].

Due to all above reasons, it is essential to run multiple model simulations using varying input feature values and feature combinations to achieve the robust output by eliminating over fitting errors [1]. This requires high computational power and time intensive simulation runs. Introduction of parallel computing techniques has overcome this issue by parallelizing the number of simulations runs in an acceptable time frame [2]. However, High performance computing (HPC) and Cloud Computing are difficult to reach by environmental scientists due to lack of programming knowledge in parallel computing and lack of hardware resources. Therefore, this research demonstrates the development, applications and performance of low-cost 4-node Single Board Computer cluster for environmental modelling by parallelizing tasks through MPI (Massage Passing Interface).

The Single Board Computer (SBC) is a complete computer build on a single circuit board, with microprocessor(s), memory, Input/output (I/O) and other features required for a functional computer [3,4]. The Raspberry Pi is a fully featured credit card sized SBC, which is capable of running programs as a standard personal computer (PC). Since its launch in 2012, the Raspberry Pi has become one of the best-selling SBC across both industry and academia [3]. In March 2017, it becomes the third best-selling general-purpose computer of all time [3]. Most recent versions of Raspberry Pi family, Raspberry Pi 3 B+ (RPiB3+) was selected for this study. Overview of the RPiB3+ SBC is shown in the Table 1.

Table 1. Overview of RPi3B+

Property	Details
System on Chip (SoC)	BCM2837B0
Cores/ Clock	4 X 1.4 GHz
Architecture	64 bits
GPU	Broadcom VideoCore IV
RAM	1 GB
Input/ Output	AV, Bluetooth, WIFI, CSI, DSI, Gigabit Ethernet, GPIO, HDMI, I2C, I2S, MicroSD, PoE Header, SPI, USB2

Materials and Methods

Table 2. shows the components used for the Pi cluster with their cost to demonstrate how economically feasible to build a small computer cluster using SBCs even for the personal use.

Table 2. Component used for Pi Cluster with their cost (Cost by date: 2018.07.25)

Component	Amount	Total Price (USD)
Raspberry Pi 3 B+	4	163.96
MicroSD 16GB	4	27.88
Micro USB cable	4	5.99
6 port USB Power Supply	1	12.36
5 port Network Switch (used)	1	9.99
Ethernet Cable	4	5.98
Other Accessories	1	6.10
Total Price		**232.26**

Figure 1. Complete Cluster setup while running

The Cluster setup is shown in figure 1. First, Raspbian Stretch with Desktop OS was installed on a MicroSD card and later configured through SSH (Secure Shell). Raspbian OS is a well-documented, timely updated widely used operating system for Raspberry Pi given by the Raspberry Pi organization. MPICH3 and MPI4Py also installed along with the updates to be able to communicate with each node and behave like one individual cluster. Once the operating system and parallel programming framework was configured for a single Pi, the operating system image was burned onto other SD cards for the rest of the nodes to avoid the repetition of the work for each node. The network configuration was established by setting static IP addresses to each node. The Pis were set up as master and slave to enable them to function as a cluster computer. One Pi was configured as the master node of the cluster, while others were configured as slaves. IP range 192.168.2.1 - 192.168.2.4 are used for the cluster to eliminate the confusion with IP addresses assigned by the router. Master node was labeled as Pi01 at 192.168.2.1 and the other nodes as Pi02 at 192.168.2.2 then Pi03 at 192.168.2.3 and so on. In this way, it is easy to remember each node with their corresponding IP. After the static addressing, it was possible to login to each node, though Pi01 (master node), but it requires entering the password of each node in every time. Therefore, the master node should have access to other slave Pis over SSH without entering the password and this was achieved by generating SSH keys for each Pi and sharing the keys of each slave node with the master node. The master node's key was added to the list of authorized keys for each of the slave nodes and the keys of the slave nodes was added to the authorized keys list of the master node. This ensured that the slave nodes could talk to the master node at will and vice versa.

The Cluster was used to compute some complex environmental models by parallelizing the task with MPI4Py and Rmpi using Python and R languages [5]. But, to demonstrate the performance of the cluster in terms of execution time, the calculation of pi (π) using Monte Carlo method was used.

Results and Discussion

The Raspberry Pi cluster shows very promising results with the tests conducted on them. Table 3 compares the time taken by different number of nodes to calculate π (pi) with 1.2×10^8 number of tosses. The times are in seconds and are averages of 10 runs.

Table 3. Performance of pi calculation using Monte Carlo method on cluster nodes.

Number of Nodes	Time Taken (Seconds)
1 (Pi01)	18.06
2 (Pi01, Pi02)	10.11
3 (Pi01, Pi02, Pi03)	6.05
4 (Pi01, Pi02, Pi03, Pi04)	5.02

Several disadvantages also observed compared to a traditional cluster. These include; 1) The need for architecture specific compilers (armv71). 2) Limited computing resources per node (memory, CPU speed, storage). and 3) Increased hardware failure rate. Some of these disadvantages can be overcome by increasing the number of nodes in the cluster.

Conclusions and Recommendations

The main goal of this research was to demonstrate the feasibility of building low-cost cluster computer for environmental modelling. It was able to build a fully functional 4-node Raspberry Pi 3 Model B+ cluster computer less than 250 USD and was able to run the predictive modelling using R and python. Calculation of π using Monte Carlo simulation in python demonstrates that the Raspberry Pi cluster is a very promising alternative to cluster computers commonly used in research and academia. Researchers who run the predictive modelling in environmental sciences can build their own computer cluster with affordable cost to save their time for running long statistical calculations with large number of iterations. This approach will be both fun and extremely educational for those who are new to the parallel programming and cluster computing. They can get a thorough knowledge on cluster computing through a 'learning by doing' approach before working with commercial HCP clusters.

References

[1] Rahn, K., Klatt, S. and Butterbach-Bahl, K., "Improving efficiency of a statistical analysis of complex ecological models, when using the statistical software R by parallelizing tasks with Rmpi." Ecological Informatics, Vol. 15: 53-57, 2013.

[2] Foster, I. T., "Designing and Building Parallel Programs" Addison-Wesley Publishing Company, pp. 379, 1994.

[3] Johnston, S. J. et al., "Commodity single board computer clusters and their applications." Future Generation Computer Systems, 89: 201-212, 2018.

[4] Raspberry Pi Foundation, Raspberry Pi 3 Model B+, 2018. https://www.raspberrypi.org (Accessed 12 September 2018).

[5] Schmidberger, M. et al., "State of the Art in Parallel Computing with R." Journal of Statistical Software, 31 (1), 2009.

GAS SENSING PROPERTIES OF HEMATITE ($\alpha - Fe_2O_3$) NANOSTRUCTURE BINDERED WITH PTFE

H.T.D.S. Madusanka*[1, 2], G.G. Chathuranga[1, 2], W.A.D.P.B. Senarathne[2], P. Samarasekara[3], U.S. Liyanaarahchi[2], C.A.N. Fernando[2]

[1]Department of Electronics, Faculty of Applied Sciences, Wayamba University of Sri Lanka, Kuliyapitiya
[2]Nanotechnology Research laboratory, Department of Nano Science Technology, Faculty of Technology, Wayamba University of Sri Lanka, Kuliyapitiya,
[3]Department of Physics, University of Peradeniya, Peradeniya, Sri Lanka.
**Corresponding author (email: dinu.sri.m@gmail.com)*

Introduction

There has been a fast growing concern for environmental safety regarding the extensive use of toxic gases. Therefore, the demands for accurate and fast detection of these type of gases had been increased. In particular, detection and monitoring of NH_3 are more demanded for the fields of environmental protection, chemical industry, medical diagnostic, explosive detection and food freshness monitoring. When it comes to the detection of materials for toxic gases, various metal oxide semiconductors such as SnO_2, ZnO, Fe_2O_3, and TiO_2 gained much attention due to their high sensitivity, selectivity and fast response [1,2]. Anyhow, most of metal oxide gas sensors operated at elevated temperatures. This drawback is much unfavorable for designing low cost and low power consuming gas sensors. Therefore, a trend is surpassing to investigate new ways to develop gas sensors more performable at Room Tempereture (RT) with respect to an economical aspect.

Hematite ($\alpha - Fe_2O_3$) is an earth-abundant, low-cost semiconductor with n-type material characteristics [3]. It is widely used in the detection of toxic gases, and many studies carried out to find its gas sensing mechanism due to its attractive performances [4,5].

Polytetrafluoroethylene (PTFE) is a binder which is commonly used to bind small particles together. Usually the use of polymer binders in thin film fabrication is a standard to adhere the film to substrate properly. Anyhow, few recent studies have shown that the combination of metal oxide and polymer binder are able to provide RT working condition [6,7].

In our study, properties of economically fabricated PTFE bindered α—Fe_2O_3 gas sensors' are presented. Its gas sensing properties were analyzed in presence of NH_3, acetone vapor and methanol vapor at RT. Furthermore, the PTFE binder

effect to the thick film structure modification and ability to alter the typical metal oxide gas sensing mechanism with respect to gain RT working feature were investigated.

Materials and Methods

All the chemicals used were an analytical grade, purchased from Sigma Aldrich. α– Fe_2O_3 powder was prepared by oxidation of iron acetate at 600 °C for one hour, 10 °C/min heating rate. Nanostructured α – Fe_2O_3 was synthesized by dissolving a certain amount of α – Fe_2O_3 powder with PTFE (20% based on the weight ratio). Distilled water was used as the solvent and mixture was stirred for one hour at room temperature to make the final solution.

The gas sensors were fabricated by drop casting the above synthesized PTFE bindered α – Fe_2O_3 solution on well cleaned Fluorine doped Tin Oxide (FTO, 15 Ω per sq.) plates which were having a 4 mm wide cut to separate two electrodes. The drop cast thick films were dried in air for 15 min, and subsequently annealed at 300 °C for one hour at ambient atmosphere with 10 °C/min heating rate.

The gas sensing measurements were carried out in a custom-ebuilt static gas sensing characterization system. The gas sensor was mounted on the heating plate fixed in the test chamber. Known gas concentrations from NH_3, C_3H_6O and CH_3OH were injected into the glass chamber through a syringe. The temperature and humidity were maintained at 27 °C and 65 %, throughout the measuring period for RT condition.

The sensor response (S) is defined as,

$$S(\%) = \frac{R_a\text{-}R_g}{R_a} \times 100\%,$$

Where R_a and R_g are the sensor electrical resistances in ambient air and in measuring gas atmosphere, respectively. The resistance measurements were carried out using Keithley 6400 Source Meter Unit. The UV – visible absorption spectra of α – Fe_2O_3 films were measured using Shimadzu 1800. Fourier Transform Infrared (FTIR) spectrums of prepared films were carried out using Shimadzu IRAffinity-1S. Surface morphology of the films was analyzed using Zeiss Evo LS15 Scanning Electron Microscope (SEM). X-ray diffraction (XRD, Rigaku Ultima IV) was used to identify the phase structures with the help of PDF2 database from the International Center for Diffraction Data (ICDD). The XRD was operated at a voltage of 40 kV and a current of 30 mA using CuKα (λ = 1.5405 A°) radiation.

Results and Discussion

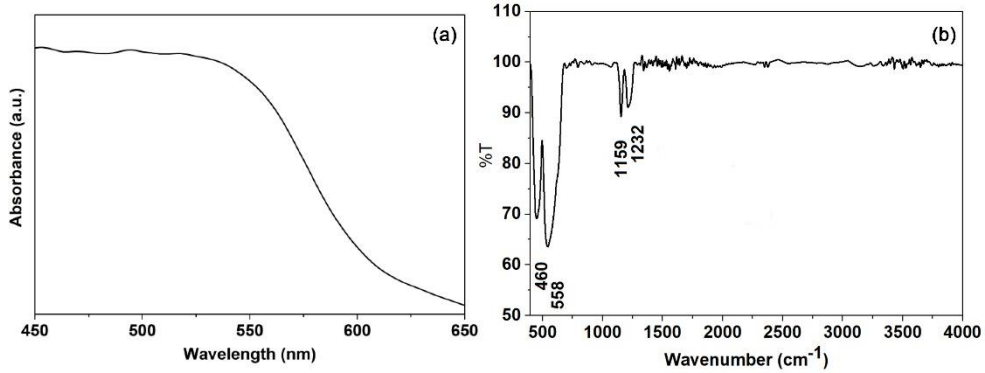


Figure 1. (a) UV-Visible diffuse reflectance spectra and (b) FTIR spectrums of PTFE bindered α – Fe_2O_3 thick film

Figure 1 (a) shows UV-Visible absorption spectra of the nanostructured PTFE bindered α – Fe_2O_3 thick film drop cast on a glass substrate. The curve exhibits an absorbance onset around 600 nm which is consistent with the band gap of α – Fe_2O_3 (~ 2.1 eV) [7]. The chemical structure of fabricated samples was analyzed using FTIR spectroscopy and displayed in Figure 1 (b). The two bands appear at 1159 and 1232 cm^{-1} are due to the stretching vibration of C-N from PTFE binder. The sharp peaks at 460 and 558 cm^{-1} correspond to the Fe-O bond stretching, which confirm the presence of α – Fe_2O_3 and PTFE binder in the samples.

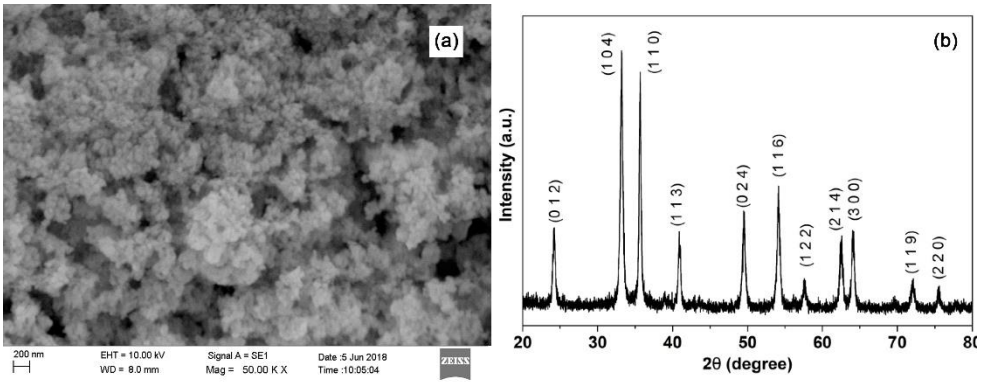


Figure 2: (a) SEM image and (b) XRD patterns of PTFE bindered α – Fe_2O_3 thick film

Surface morphology of PTFE bindered α – Fe_2O_3 were analyzed using SEM and displayed in Figure 2(a). SEM micrograph clearly shows spherical shaped interconnected α – Fe_2O_3 grains with 80 nm average diameter size. Further, it indicates a higher porous morphology. The combination of nanoscale grains with porous morphology is very much preferred for efficient gas sensing applications due to its high surface to volume ratio which promotes the adsorption of gas molecules through the sensor surface. Figure 2(b) shows the XRD pattern of hematite (ICDD 33-0664) and in agreement with the hexagonal structure of α – Fe_2O_3. The peaks observed at 2θ values of 24.16°, 33.18°, 35.65°, 40.88°, 49.5°, 54.17°, 57.57°, 62.47°, 64.01°, 75.53° and 77.81° possesses orientations in the (012), (104), (110), (113), (024), (116), (122), (214), (300), (119) and (220) planes, respectively. The narrow sharp peaks indicate that fabricated α – Fe_2O_3 thick films are highly crystalline.

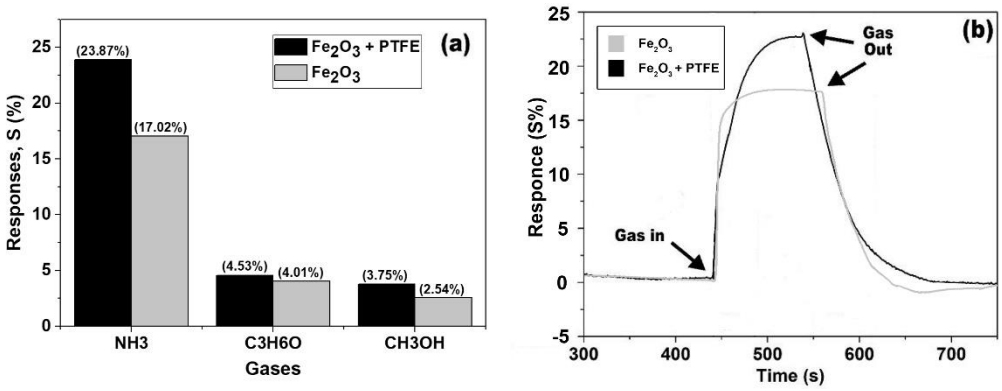


Figure 3. (a) Gas response of nanostructured PTFE bindered α – Fe_2O_3 sensor and pure α – Fe_2O_3 sensor to 1000 ppm of NH_3, acetone vapor and methanol vapor at RT, (b) The response time and recovery time of the PTFE bindered α – Fe_2O_3 sensor and pure α – Fe_2O_3 sensor to 1000 ppm NH_3 at RT

Figure 3(a) shows the gas response bar diagram for three different gases at RT with a known concentration of 1000 ppm. According to the bar diagram, the PTFE bindered α – Fe_2O_3 sensor exhibits a higher response to NH_3 (23.87%) than to acetone vapor and methanol vapor. It reveals that the fabricated sensor is more selective towards NH_3 than to other tested gases.

Moreover, the response time and recovery time are other important parameters in characterizing a gas sensor. Fig. 3(b) show the response of the sensor at 1000 ppm NH_3 exposure in RT. The response and recovery times for PTFE bindered α – Fe_2O_3 sensor were 45 and 66 s, respectively. Similarly, for pure α – Fe_2O_3, the values were 31 and 60 s. The Pure α – Fe_2O_3 gas sensor exhibits relatively fast response and recovery times compared with PTFE bindered α – Fe_2O_3 gas sensor.

Even though the mechanism of metal-oxide based gas sensors was reported in many literatures [8,9], yet the gas reaction mechanism of polymer bindered metal-oxide sensors was not well explained. As suggested in this study [10], it can be due to the complex chemistry that changes the sensing material which is consist of not only metal-oxides, but also polymer binders. Normally, metal-oxide based gas sensors tend to show their maximum sensitivity at higher temperatures. It had observed that by introducing conducting polymer into metal-oxide based gas sensors, their ability to give good sensitivity at RT is enhanced.

The most of polymer binders like PTFE contain an electrically insulating polymer matrix. When a sensor material is fabricated by combining metal-oxide like sensing materials with a polymer binder, the resultant composite resistivity mostly depends on the effective surface volume. Also the permeation of testing gases into the sensing structure can lead to swelling the polymer matrix and hence further increased the effective surface area. Besides that, this process can increase the distance between metal-oxide nano particles' grain boundaries. Therefore, it may result to induce additional electrical resistance value into the sensor other than from surface adsorbed gas molecules [11]. This added resistance together with the resistance from physisorption can be the reason for the observed high sensitivity at RT. The same reason can be used to explain the delayed response time and delayed recovery time exhibited in PTFE bindered α – Fe_2O_3 sensor with respect to the extra time taken to swell the polymer matrix. Anyhow, the sensors' sensitivity reduced rapidly with the increase of the temperature. Even though α – Fe_2O_3 particles are able to give good sensitivity at high temperature, the PTFE polymer binder in the composite cause rapid reduction of its electrical conductivity resulting overall low sensitivity at elevated temperatures. These observations match with several other recent research works based on polymer bindered metal-oxide gas sensors [11,12].

Conclusions and Recommendations

PTFE bindered nanostructured α – Fe_2O_3 thick film gas sensor was fabricated and characterized. The PTFE bindered α–Fe_2O_3 gas sensor exhibits good sensitivity towards NH_3 gas at RT with fast response time. It was found that gas sensing mechanism as a combination of the swelling process of PTFE polymer binder and oxygen ion induced majority carrier modulation in the metal oxide particles. It can be suggested that highly sensitive RT operating conditions can be achieved with the help of conducting polymer binders. This approach is much favorable in developing a room temperature economical gas sensor for future gas sensing applications.

References

[1] K. Arshak, M. Nicholson, A. Arshak, E. G. Moore, I. Gaidan, and C. Cunniffe, "Examining the effects of polymer binder in Fe2O3/ZnO thick film sensors on the response to propanol vapour," in 2006 29th International Spring Seminar on Electronics Technology, 2006, pp. 489–494.

[2] D. Garcia, G. Picasso, P. Hidalgo, H. E. M. Peres, R. Sun Kou, and J. M. Gonçalves, "Sensors based on Ag-loaded hematite (α-Fe2O3) nanoparticles for methyl mercaptan detection at room temperature," Anal. Chem. Res., vol. 12, pp. 74–81, 2017.

[3] S. T. Navale et al., "Synthesis of Fe2O3nanoparticles for nitrogen dioxide gas sensing applications," Ceram. Int., vol. 39, no. 6, pp. 6453–6460, 2013.

[4] S. Wang et al., "Porous α-Fe2O3hollow microspheres and their application for acetone sensor," J. Solid State Chem., vol. 183, no. 12, pp. 2869–2876, 2010.

[5] K. . Arshak, M. Nicholson, A. Arshak, E. G. Moore, I. Gaidan, and C. Cunniffe, "Examining the effects of polymer binder in Fe2O3/ZnO thick film sensors on the response to propanol vapour," in 2006 29th International Spring Seminar on Electronics Technology, 2006, pp. 489–494.

[6] K. I. Arshak, L. M. Cavanagh, I. Gaidan, E. G. Moore, and S. A. Clifford, "Influence of the Poly (Vinyl Butyral) Binder in Screen-Printed Thick-Film Gas Sensors Containing NiO - TiO 2 Oxides," IEEE Sens. J., vol. 7, no. 6, pp. 925–930, 2007.

[7] W. D. Chemelewski, O. Mabayoje, D. Tang, A. J. E. Rettie, and C. Buddie Mullins, "Bandgap engineering of Fe 2 O 3 with Cr – application to photoelectrochemical oxidation," Phys. Chem. Chem. Phys., vol. 18, no. 3, pp. 1644–1648, 2016.

[8] J. Zhang, Z. Qin, D. Zeng, and C. Xie, "Metal-oxide-semiconductor based gas sensors: Screening, preparation, and integration," Phys. Chem. Chem. Phys., vol. 19, no. 9, pp. 6313–6329, 2017.

[9] Y. F. Sun et al., "Metal oxide nanostructures and their gas sensing properties: A review," Sensors, vol. 12, no. 3, pp. 2610–2631, 2012.

[10] I. Gaidan, D. Brabazon, and I. Ahad, "Response of a Zn2TiO4 Gas Sensor to Propanol at Room Temperature," Sensors, vol. 17, no. 12, p. 1995, 2017.

[11] K. I. Arshak, L. M. Cavanagh, I. Gaidan, E. G. Moore, and S. A. Clifford, "Influence of the Poly (Vinyl Butyral) Binder in Screen-Printed Thick-Film Gas Sensors Containing NiO - TiO 2 Oxides," IEEE Sens. J., vol. 7, no. 6, pp. 925–930, 2007.

[12] J. N. Gavgani et al., "A room temperature volatile organic compound sensor with enhanced performance, fast response and recovery based on N-doped graphene quantum dots and poly(3,4-ethylenedioxythiophene)–poly(styrenesulfonate) nanocomposite," RSC Adv., vol. 5, no. 71, pp. 57559–57567, 2015.

PILOT SCALE LANDFILL LEACHATE TREATMENT SYSTEM USING TREATMENT TRAIN COMPRISED ANAMMOX, BIOCHAR BARRICADES AND CONSTRUCTED WETLAND

M. Vithanage[2], B.C.L. Athapattu[1], Sarangi Joseph[1], Dilsharan Bagawan[1], Nadeesha Punchihewa[1]

[1]Department of Civil Engineering, Faculty of Engineering Technology, The Open University of Sri Lanka, Nawala, Sri Lanka
[2]Ecosphere Resilience Research Center, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
**Corresponding author (email: sarangi.madhavij@gmail.com)*

Introduction

Leachate generation from municipal landfills and open dumps has become an environmental problem as it contains high concentration of organics, ammonia, nutrients, heavy metals, and other pollutants. A land in wetlands of Boralesgamuwa – Borupana is being used as an open dumping pit and known as Karadiyana garbage dump. It is situated adjacent to the 'Weras Ganga', which flows into Bolgoda Lake [1]. Waste collected from various municipal council and urban council areas has been dumped to this site for more than 35 years. The amount of waste disposed at this site is around 400-500 Mt per day [2]. This landfill leachate is a vital environmental problem which pollutes ground and surface water bodies in the area. Leachate treatment with cost effective and robust technologies is thus a major engineering challenge. Given the highly variable and multi-faceted nature of leachate, no single unit process is adequate, a combination of diverse unit processes working in conjunction is necessary.

Biochar has been shown to have adsorptive properties similar to activated carbon. A preliminary investigation on the use of biochar generated from the organic fraction of municipal solid waste (MSW) for controlling trace organics in leachate was presented. This work includes the study of mechanisms of Municipal Solid Waste Biochar (MSW-BC) and gasifier wood biochar to assess their potential in treating leachate from open dumps by using biochar column reactors.

Our aim is to design and construct a leachate treatment system at Karadiyana garbage dump to treat leachate. This work will involve to test the leachate treatment at laboratory level and select the best operational conditions for the treatment system using anammox bacteria, constructed wetland and biochar reactor.

Materials and Methods

Determination of leachate discharge

A v – notch was constructed on across leachate surface flow path on a selected area to measure leachate discharge and to measure its fluctuation with rainfall. Study area was selected by meticulous study around the garbage dump considering topography as well as leachate path which maximum flow may occur.

Biochar production using MSW

Biochar production is being carried out at the Dump site. Initially, biochar was produced using char drum oven. It was made by two 200 liters steel barrels and two 60-liter steel barrels as shown in figure 1. One larger one was used as pyrolyzer and filled with flammable materials such as dried MSW, fire wood. It was placed on well firmed ground and top of the drum covered with steel plate leaving 300mm hole so that heat can be transferred above.

One small drum filled with MSW which need to be turned in to biochar. It was well compacted so that lower the oxygen content and properly covered as well as small holes were drilled around the top edge so that facilitate to escape vapors. Chimney was made out with small drum and fixed on top to facilitate to emission. The pyrolyzer drum was allowed to burn for three hours. Woods were inserted to the pyrolyzer drum with the time. With the completion of burning, water was poured to cooldown the drums.

Figure 1. Biochar pyrolyzer

Preparation of biochar reactor

Biochar were tested to determine the removal efficiency of pollutant of leachate samples collected at site. Leachate samples were collected few hours before the test. Anaerobic digester was used to test biochar samples as shown in fig 2. Samples was prepared by disintegrating biochar into particle size range less than 5mm to larger than 1.18mm and laterite sample was also prepared with particle range 3.5mm-14mm. Biochar prepared with MSW was packed inside the left-hand side column of the digester. It was packed layer by layer so that sandwich

Figure 2. Biochar column set-up at the laboratory

with laterite layers as well as at a ratio 2:1 in terms laterite: biochar weight ratio. The flow rate is adjusted to 1.5 ml/min for MSW biochar & 1 ml/min for wood biochar. In this experiment first wood biochar tested to identify the removal pollution. Then MSW biochar was tested.

Biochar micro column test

Rapid Small-Scale Column Tests will be conducted to quantify sorption of metals, organics, and nutrients from leachate by biochars made from wood and MSW compared with GAC. Initially biochar will be sieve by 200 mesh (75 micro meter sieve size) and compact in a small column. Initial parameters will be tested under this experiment in order to identify suitable biochar.

Growing Anammox

The anammox will be inoculated with granular anammox sludge from a full-scale anammox reactor at the HRSD York River Treatment Plant. This biomass will be used as a culture. Anaerobic digester will be used to enrich anammox in lab scale at OUSL laboratory.

Designing Leachate Collection System & complete treatment plant

A leachate collection system (drainage network) will be designed to collect all leachate to treatment basin. Further a treatment plant will be designed to treat all leachate collect from the dump

Construct the treatment process & Testing the effluent

The treatment process will be constructed at site to treat the leachate. Laboratory tests will be carried out to identify the concentration of pollutants (pH, EC, BOD, COD, Total Solids, nutrients; nitrate, phosphate, nitrite, ammonia, etc., anions; chloride, sulfate, etc., heavy metals and organic compounds; TOC, VOCs, etc. in influent and effluents.

Results and Discussion

Figure 3 shows how the leachate discharge changes with rainfall. Dry days show low discharge and discharge increases during rainy days.

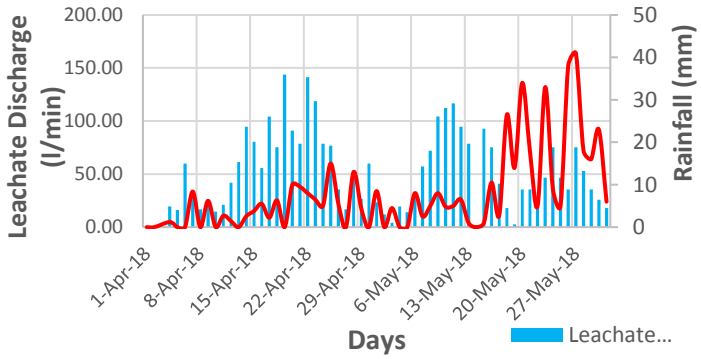


Figure 3. Variation of Leachate discharge & Rainfall from April 2018

It could be seen that the leachate discharge varies from 2.4 l/s to 0.02 l/s in rainy to dry period respectively. Maximum leachate shows when the rainfall is maximum, 8 mm. Landfill leachate quality were influenced from the waste composition, the age of landfill, waste amount, rain intensity as well as the category of municipal landfill solid waste [3]. However, the generation of leachate quantity is quite high in the rainy days with compare to dry period.

Table 1. Leachate quality in Karadiyana Open Dump

Parameter	Value
pH	8.46
COD (mg/l)	8060
BOD (mg/l)	715
TOC (mg/l)	2334
Ammonia (mg/l)	972.70
Nitrate (mg/l)	6.52
Nitrite (mg/l)	Not Detected
Phosphate (mg/l)	3.15
Sulphate (mg/l)	57.03
Iron (mg/l)	19458.70
Lead (µg/l)	537.93
Chromium (µg/l)	883.64
Manganese (µg/l)	1151.14
Zinc (µg/l)	10370.64
Nickle (µg/l)	497.27

(Source: Yohan, 2018)

The leachate composition indicates in table 1. It can be seen that trace metals, such as Cu, Ni, Cd, Zn, Pb, and Mn, are at slightly higher concentrations in raw leachate.

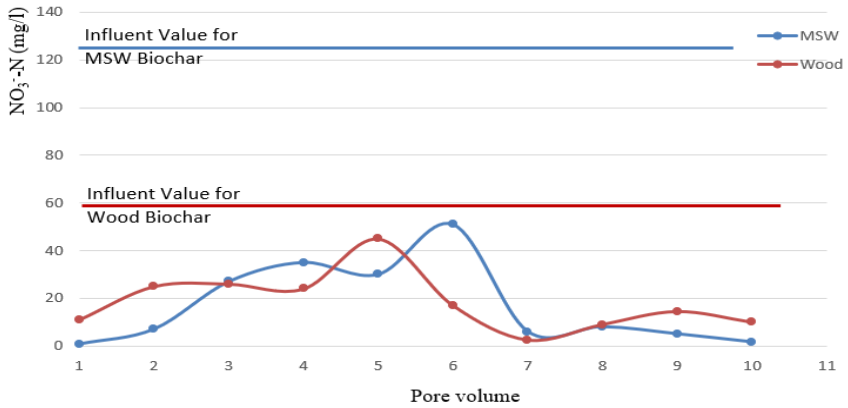


Figure 4. Reduction of NO_3^--N of leachate in MSW & Wood biochar reactors

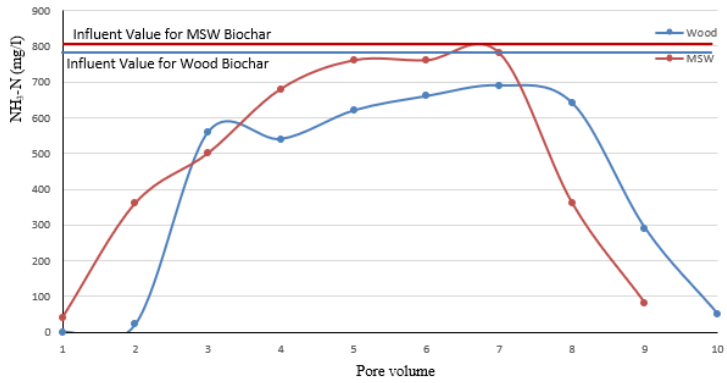


Figure 5. Reduction of NH_4--N of leachate in MSW & Wood biochar reactors

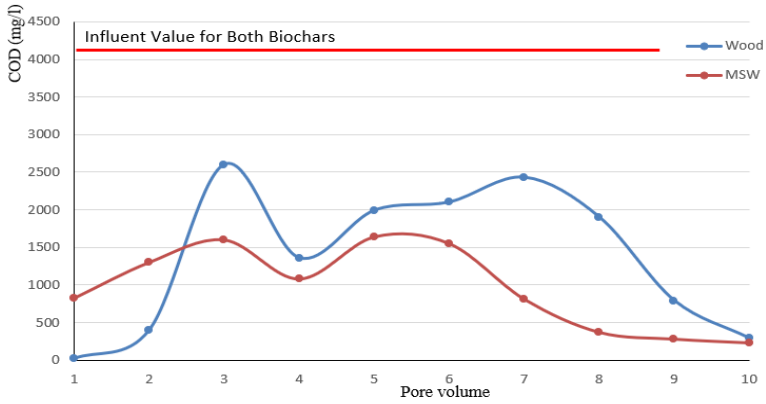


Figure 6. Reduction of COD of leachate in MSW & Wood biochar reactors

It can be concluded that the continuous flow of leachate through biochar significantly effect for reducing contaminant. Initially each pollutant has been decreased considerably, with the time pollutant removal efficiency is decreased.

Since this test was done for MSW biochar and Wood biochar in separate time period, the influent of leachate parameters has been changed. The pollutant removal efficiency is given in table 2.

Table 2. Removal efficiency of pollutants through bio char reactors

		Removal efficiency g/Kg	
Parameters	Unit	Column 1 (MSW Biochar)	Column 2 (Wood Biochar)
COD	g COD/kg	29.972	27.794
NH_3-N	g NH_3-N/kg	2.0963	3.815
NO_3^-N	g NO_3^--N/kg	0.683	0.397

Conclusion

Leachate found to be high in many parameters including ammonia. Wood-biochar displayed relatively high adsorption capacity to 1.719 mg/kg ammonia than MSW, while MSW shows high adsorption capacity to 0.286 mg/kg nitrate & 2.178mg/kg COD than wood biochar. According to the results both gasifier wood biochar and MSW biochar removed pollutant considerably, thus both can be used as pollutant removal absorbent in leachate.

References

[1] DAS Environmental Expert GmbH, Dresden. (2018) Processes for Landfill Leachate Treatment in Landfills and Sewage https://www.das-ee.com/en/wastewater-treatment/industries/landfill-leachate/ (accessed 31/03/2018)

[2] Environmental Foundation Limited (EFL). (2016) karadiyana garbage dump http://efl.lk/karadiyana-garbage-dump/ (accessed 16/02/2018)

[3] Zakaria, S. N. F. & Aziz, H. A (2018) Characteristic of leachate at Alor Pongsu Landfill Site, Perak, Malaysia: A comparative study. The journal of Earth & environmental science Volume 1, pp.140

[4] Yohan, Y. (2018), test results, Leachate quality in karadiyana open dump site, National Institute of Fundamental Studies, Kandy, Sri Lanka.

BENTHIC MACRO INVERTEBRATE DIVERSITY INDICES TO ASSESS THE WATER AND SEDIMENT QUALITY IN DANDUGAN OYA, SRI LANKA

L.G.Y.J.G. Wadasinghe and W.M.D.N. Wijeyaratne*

Department of Zoology and Environmental Management, University of Kelaniya, Kelaniya, Sri Lanka
**Corresponding author (email: dimuthu.wijeyartne@kln.ac.lk)*

Introduction

Benthic macro-invertebrates are very sensitive to the organic and inorganic pollutants. Therefore, the diversity of benthic macroinvertebrates is a major biological parameter that can be used in biomonitoring of aquatic ecosystems [1]. The biological data on diversity of benthic macroinvertebrates can be used to calculate biological indices that can predict the water quality and pollution status of river ecosystems [2].The present study was conducted in the Dandugan Oya, which is a stream located in the western province of Sri Lanka and is receiving industrial waste from multiple point and non-point sources. It also serves as a raw water source for public water supply in some suburban areas in the Gampaha District. In the present study, the macro-benthic ivertebrate diversity indices were used to assess the water and sediment quality of the Dandugan Oya.

Materials and Methodology

Shallow water and sediments (0-0.4 m depth) were collected from six sites (Site A: urban site; B and D: Industrial sites; C: water intake for public water supply; E: agricultural site; F: reference site with pristine environmental conditions) at two month intervals from May to November 2017 (Figure 01). Water quality (pH, conductivity, total dissolved solids, temperature, dissolved oxygen (DO), nitrate, phosphate, salinity, chemical oxygen demand (COD) and biological oxygen demand) and sediment quality (pH, conductivity, total organic carbon content and sediment texture class) was analysed using standard analytical methods. The data on diversity of benthic macro-invertebrates were used to determine diversity indices (Simpson's Diversity Index, Shannon-Wiener Diversity Index, Pielou's Evenness Index, Ratio of Ephemeroptera, Plecoptera, and Trichoptera (EPT) to Chironomidae abundance (EPT/C) and Family Biotic index (FBI)), species richness and total abundance. Spatial variation of water and sediment quality and abundance of macro benthic invertebrates were analysed by ANOVA followed by Tukey's pairwise comparison and Principal Component Analysis (PCA) on water and sediment quality parameters were performed using MINITAB 14 software.

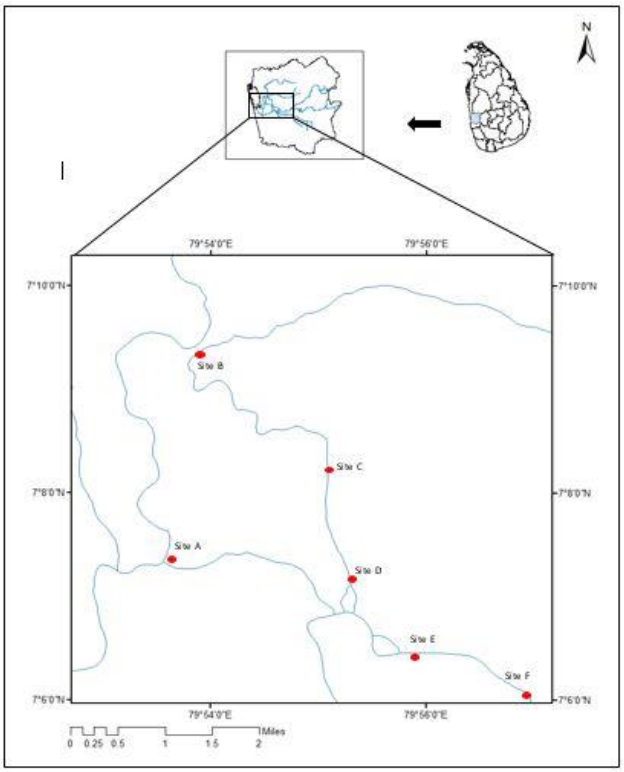


Figure 1. Map of the study area showing sampling sites

Results and discussion

Water quality and sediment quality parameters

Significant spatial variations of water quality and sediment quality parameters were observed. Significantly high water pH (6.9), conductivity (4457 µS cm^{-1}) salinity (2.44 $^{0}/_{00}$) and TDS (2375 mg L^{-1}) were recorded in site A. Significantly lower DO was recorded from sites C (4.0 mg L^{-1}) and D (4.3 mg L^{-1}) and significantly high COD was recorded from site D (267.1 mg L^{-1}). In sediment quality, significantly lower % sand content (37%) and significantly higher % silt content (44%) was recorded from site B. Significantly higher % clay content (40.6%) was recorded from Site D. pH of all the sites indicated slightly acidic sediments. Significantly lower sediment pH (4.71) and sediment conductivity (25.29 µS cm^{-1}) were recorded from the reference site (Site F). Highest total organic matter (2.32 %) content was recorded from the urban site (Site A). According to the PCA all the sites are significantly differ from each sites except site E and F.

Benthic Macro-invertebrates

Spatial variation of diversity and biotic indices of benthic macro-invertebrates are given in Table 1. Site B and site E showed the highest species richness. Simpson's Diversity Index, Shannon-Wiener diversity Index and Pielou's Eveness Index of site

E showed higher values than those of other sites. Site D recorded a lower EPT/C value and higher Family Biotic Index that indicated a poor biotic condition and poor water quality than that of the other sites. Most sites were dominant by members of the Family Chironomidae, whereas site A was dominated by members of the Oder Tricoptera. There was no significant spatial variation of the abundance of benthic macro-invertebrates during the study period except in members of Family Thiaridae. According to the PCA on water and sediment quality parameters (Figure 2), Sites E and F were grouped together and all the other sites were separated from each other. There were liner positive relationships with the PC Score 1 values of water and sediment quality parameters with Simpson's Diversity index, Shannon-Weiner Diversity Index and Pielou's Evenness Index (p-value < 0.05). There were negative relationships with the PC Score 1 values of water and sediment quality parameters with total abundance and EPT/C ratio (p-value < 0.05). The R^2 indicated that, EPT/C ratio was responsible for 68.79 % of the variation in PC Score 1.

Table 1. Diversity and Biotic Indices of benthic macro invertebrates among sampling sites during the study period

Site	Total abundance	Simpson's Diversity Index (D)	Shannon-Wiener Diversity Index (H')	Pielou's Evenness Index	EPT/C ratio	FBI value
A	462	0.524	1.115	1.234	3.975	2.879
B	802	0.568	1.121	1.109	0.583	5.56
C	239	0.577	1.058	1.171	0.931	4.937
D	661	0.580	1.282	1.282	0.334	6.638
E	204	0.759	1.718	1.649	0.537	5.157
F	446	0.584	1.143	1.469	0.513	6.141

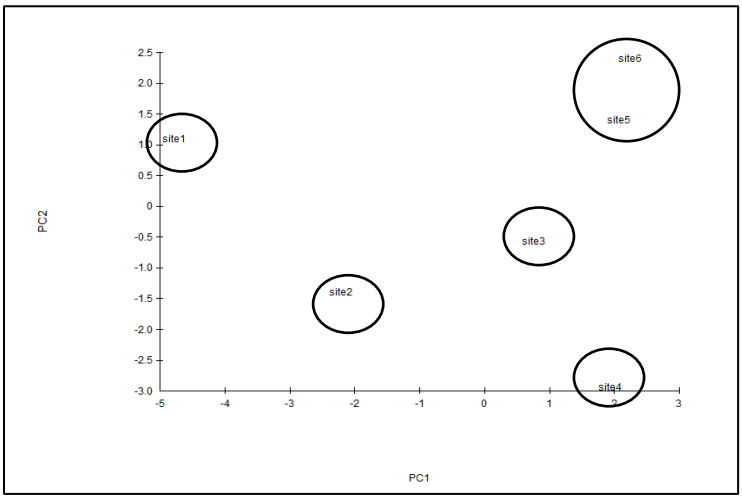


Figure 02. The Ordinance of the sampling sites in different land use areas of the PC1 and PC2 scores of Principal Component Analysis created on physico-chemical parameters of shallow water and shallow sediment qualities

Conclusions and recommendations

The results of this study revealed that the each sampling site in the Dandugan Oya is significantly characterized by the different pollution levels except agricultural site (site E) and the reference site (site F). In the present study, the industrial site D recorded significantly high percentage silt, clay contents, COD values and displayed significantly low EPT/C ratio and high FBI value. Therefore, considering the FBI value and the EPT/C value industrial site D show higher degree of pollution level compared to other sites. Results of the regression analysis indicated that EPT/C index of benthic macro-invertebrates is the most suitable diversity index to predict the water and sediment quality in Dandugan Oya.

References

[1] H. Mahushanka, H. Asanthi and R. Maithreepala, "The benthic macro invertebrate diversity in ralation to water quality of stream habitat at Dediyagala, Sri Lanka," *Sri Lanka Journal Aquatic Science,* vol. 19, pp. 15-29, 2014.

[2] M. Arzina, C. Yap, A. R. Ismail, S. Tan and A. Ismail, "Anthropogenic impacts on the disrtibution and bio diversity of benthic macroinvertebrates and water quality of the Langat River, Peninsular Malaysia," *Ecotoxicology and Environmental Safety,* vol. 64, pp. 337-347, 2006.

ASSESSMENT OF GROUNDWATER QUALITY USING WATER QUALITY INDEX IN KONDAVIL AREA, JAFFNA PENINSULA

M. Shanthamareen and W.M.D.N. Wijeyaratne*

Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya
**Corresponding author (email: dimuthu.wijeyaratne@kln.ac.lk)*

Introduction

Jaffna peninsula mainly consists of shallow karstic aquifer. It has been very intensively used and is highly impacted and deteriorated due to anthropogenic activites. High hardness is a major issue of groundwater quality throughout the Jaffna peninsula which has the risk of causing salivary stone and urolithiasis in humans. Studies have also recorded that elevated levels of nitrate content in water could be related to the high prevalence of cancer in the gastrointestinal tract of people residing in this area [1]. Therefore, groundwater quality in Jaffna peninsula can highly influence the quality of human health, agriculture and economic and social development[2].

Systematic assessment and continuous monitoring of groundwater quality is very important in Jaffna peninsula in order to implement appropriate groundwater conservation measures. Water quality index (WQI) transform large quantities of water quality data into a single number which represents the water quality level. Thus the WQI is considered a central way to convey water quality information to policy makers and the general public in a user friendly manner[3]. The aim of the present study was to use the WQI and the Sri Lanka Standards for potable water – SLS 614: 2013 to assess the drinking water quality of the Kondavil area to characterize the water quality status.

Materials and Methods

This study focuses on selected domestic wells in the Kondavil area in Jaffna peninsula which is fed by karstic aquifer. Kondavil area belongs to Nallur Divisional Secretariat and situated 3miles from the Jaffna town. Jaffna municipal area is supplied with water pumped from wells located in Kondavil and Thirunelvely**.**

Fifteen domestic wells were randomly selected (Figure 01) and water samples with three replicates were collected from each well in May, June, October 2017, covering wet and dry seasons. Temperature, Salinity, pH, Electrical conductivity, dissolved oxygen, Total dissolved solid were measured on site using pre-calibrated multi meter (HACH / model: H940 d multi). Total hardness (TH), total phosphate, chemical oxygen demand and nitrate nitrogen were estimated in the laboratory by standard methods as prescribed by American Public Health Association (APHA).

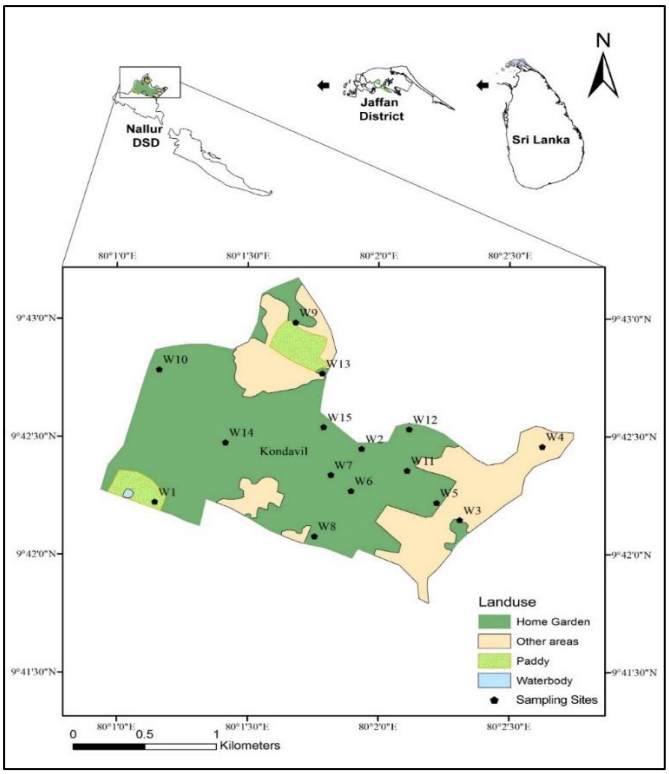


Figure 01. Map of the area showing the locations of the sampled wells

WQI was calculated by adopting the method of Reza and Singh (2010) [4] and suitability of the groundwater for drinking purposes was determined. Sri Lanka Standards for potable water – SLS 614: 2013 was referred for calculation of WQI.

Following equations were used:

First, the calculation of weightage of i^{th} parameter

$$W_i = k/S_i \qquad \text{[Equation 1]}$$

W_i - Unit weightage of i^{th} parameter, S_i - recommended standard for i^{th} parameter, k - constant of proportionality.

Second, the calculation of the quality rating for each of the water quality parameters.

$$q_i = \left(\frac{v_a - v_s}{v_i - v_s}\right) \times 100 \qquad \text{[Equation 2]}$$

q_i is the sub index of i^{th} parameter, v_a - actual value obtained from laboratory analysis of i^{th} parameter, v_i - standard value of i^{th} parameter (Sri Lankan standards), v_s = ideal value (pH=7 and 0 for all parameters)

Third, the summation of the sub-indices in the overall index

$$WQI=\frac{\sum_{i=1}^{n} q_i w_I}{\sum_{i=1}^{n} w_i} \quad \text{[Equation 3]}$$

n - Number of the parameter

Results and Discussion

From the sampled wells, five out of fifteen wells had all measured physico-chemical parameter values within the standard levels for safe drinking water. The mean TH of the sampled wells ranged from 454.8 to 784.4 mg/L $CaCO_3$ and The mean nitrate N ranged from 0.82 – 20.99 mg/L. 60% of wells exceeded the standard values for nitrate- N (11.3mg/L) and all wells exceeded the standard value for total hardness (250mg/L; SLS 614, 1983; 2013). However, the quality of the water cannot be determined based only on these two parameters. Therefore, water quality index was used to aggregate information from all the selected water quality parameters, to compare it with standard values and to give an overall single value that state the quality level of groundwater [5].

Unit weightage of parameters based on the Sri Lankan drinking water standard (SLS 614: 2013) is given in Table 1. The weightage of parameters has an inverse relationship with its permissible limits (Table 1)**.**

Variation of WQI in the sampled wells are given in Figure 2. The water quality can be categorized into 5 categories based on WQI as follows: 0-25: very good; 25.1-50: good; 50.1-75: poor; <75: very poor [4,5].

Table 1: Unit weightage of parameters based on the Sri Lankan drinking water standard (SLS 614: 2013)

Paramaeters	**Highest permitted value for water (S_i)**	**Unit weightage (w_i)**
Electrical conductivity (µS/cm)	3500	0.0003
pH	8.5	0.1176
TDS (mg/L)	500	0.0020
Total Hardness (mg/L)	250	0.0040
COD (mg/L)	10	0.1000
Nitrate-nitrogen (mg/L)	11.3	0.0885
Total phosphate (mg/L)	2	0.5000

WQI values of the sampled wells ranged from 8.99 to 36.03. Based on WQI, 80% of sampled wells were ranked as “very good” and rest of the wells were ranked into “good” category. Therefore, according to the WQI overall quality of the

ground water in the Kondavil area is good despite of having high TH and nitrate-N values higher than standard level.

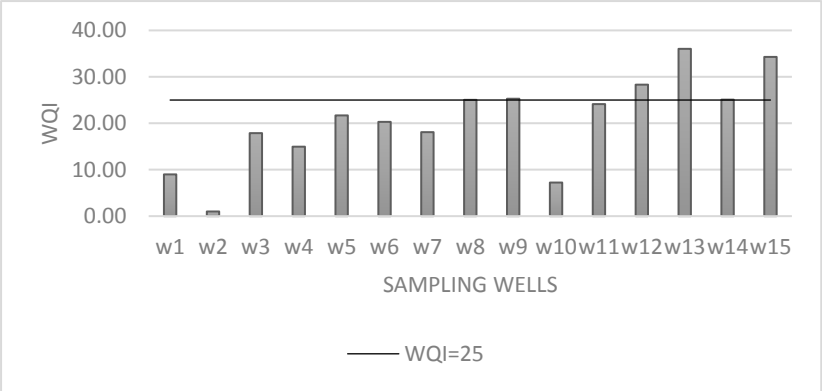


Figure 2. WQI for selected wells of the Kondavil area, black horizontal line indicate the range of good category

Conclusions and Recommendations

Based on the water quality index overall quality of the groundwater in the Kondavil area can be categorized into very good or good categories. Therefore, it can be concluded that the water in the sampled domestic wells are suitable for drinking and other domestic uses. However, continuous monitoring of nitrate- N and TH in the wells of this area is highly recommended as these water quality parameters are directly related to severe health impacts of humans.

References

[1] Sutharsiny, H. Manthrithilake, S. Pathmarajah, M. Thushyanthy, M. Vithanage "Seasonal variation of nitrate-n in groundwater: a case study from chunnakam aquifer, jaffna peninsula." *Ceylon Journal of Science (Physical Sciences)*. Vol 18, pp 1-8, 2014.

[2] C.R. Panabokke. Groundwater conditions in Sri Lanka. National Science Foundation of Sri Lanka; 2007, pp. 1-28.

[3] S. Harshan, M. Thushyanthy, J. Gunatilake, T. Srivaratharasan, K. Gunaalan. "Assessment of water quality index of groundwater quality in Chunnakam and Jaffna Town, Sri Lanka." *Vingnanam Journal of Science*. Vol 13 (1-2), pp 84 -91, 2017.

[4] R. Reza, G. Singh. "Assessment of ground water quality status by using water quality index method in Orissa, India." *World Applied Sciences Journal*. Vol 9 (12), pp 1392-1397, 2010.

[5] C. Rosu, I. Pistea, M. Calugar, I. Martonos, A. Ozunu. "Assessment of ground water quality status by using water quality index (WQI) method in Tureni village, Cluj count." *Air and Water Components of the Environment*. pp 111 – 118, 2013.

WQI AS A COMMUNICATION TOOL, TO ASSESS THE DRINKABILITY OF GROUND WATER IN CHUNNAKAM AND VADAMARADCHI, JAFFNA PENINSULA

W.M.D.N. Wijeyaratne* and S. Subanky

Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya
**Corresponding author (email: dimuthu.wijeyaratne@kln.ac.lk)*

Introduction

Ground water accounts for more than 85% safe drinking water in rural areas of most Asian countries including Sri Lanka. Lack of rain water harvesting systems and major water supply schemes caused almost 100% of water demand is to be fulfilled by groundwater in Jaffna peninsula in Sri Lanka[1]. The groundwater aquifers in Jaffna Peninsula are prone to groundwater problems such as salt water intrusion due to over extraction, sewage contamination with improper soakage pits, increased hardness and contamination with nitrate and crude oil.

The suitability of water sources for human consumption has been described in terms of Water quality index (WQI), which is one of the most effective ways to describe the quality of water. The use of water quality indices (WQIs) has the capability to reduce the bulk of the information into a single value to express the data in a simplified and logical form with different categories of water quality that reflects the overall water quality status [2]. The selection of the parameters for the index depends on several factors, such as the purpose of the index, the importance of the parameter and the availability of data. The aim of the present study was to develop WQI to monitor water quality in selected domestic wells in Chunnakam and Vadamaradchi aquifers.

Methodology

Study area

Jaffna Peninsula is fully dependent on ground water aquifers. This study focused on two Divisional Secretariat Divisions fed by two major types of aquifers in Jaffna Peninsula, namely, Vadamaradchi and Chunnakam. The Vadamaradchi area is fed by the Vadamaradchi aquifer, and is considered as the most uncontaminated aquifer in Jaffna Peninsula. Chunnakam area is fed by the Chunnakam aquifer and it is considered to be the largest aquifer in the Jaffna peninsula.

From each aquifer, three replicate water samples were collected from randomly selected 10 domestic wells (Figure 1) and preserved in accordance with American Public Health Association (APHA, 1998) until analyzing.

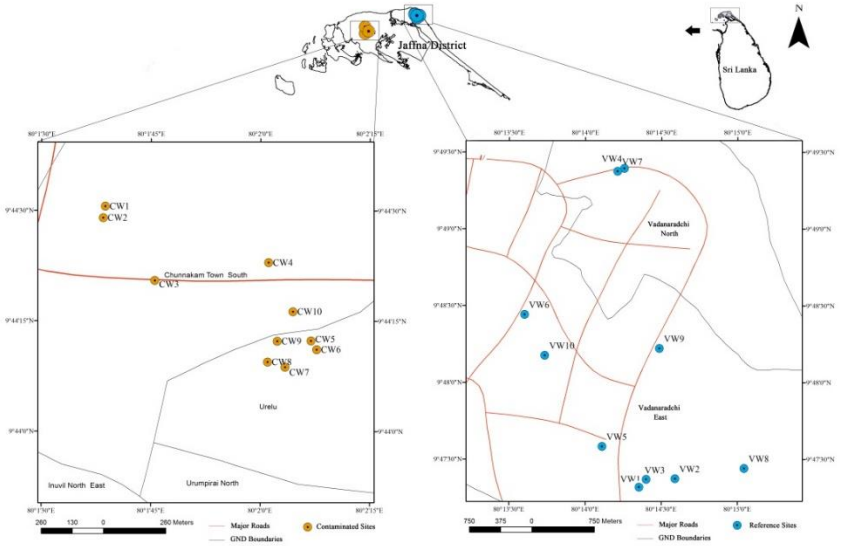


Figure 1. Locations of sampling sites

Water quality assessment and household remedial methods

The pH, Electrical Conductivity (EC) and Total Dissolved Solids (TDS) of each water sample were measured in-situ using pre-calibrated multiparameter water quality checker (HACH model: H940). Nitrate-N, Total phosphorus (TP), Total hardness (TH), Total solids (TS), Chemical oxygen demand (COD) and Oil & grease (O&G) concentration were measured following the methodologies described in APHA, 1998.

Weighted Arithmetic Water Quality Index Method

Weighted arithmetic water quality index method classify the water quality according to the degree of purity by using the most commonly measured water quality variables that affect the quality of water. The method has been widely used by the several scientists [3, 4, 5] and the calculation of WQI was made [5] by using the following equation:

$$\mathrm{WQI} = {\sum \mathrm{Q\text{-}iWi}}/{\sum \mathrm{Wi}}$$

The quality rating scale (Q_i) for each parameter is calculated by using this expression:

$$\mathrm{Qi} = \frac{\mathrm{Vi\text{-}Vo}}{\mathrm{Si\text{-}Vo}} * 100$$

Where,

V_i is estimated concentration of i^{th} parameter in the analyzed water

V_O is the ideal value of this parameter in pure water

V_O = 0 (except pH =7)

S_i is recommended standard value of i^{th} parameter.

The unit weight (W_i) for each water quality parameter is calculated by using the following formula:

$$\mathrm{Wi} = {}^{\mathrm{K}}/_{\mathrm{Si}}$$

Where,

K = proportionality constant and can also be calculated by using the following equation:

$$\mathrm{K} = \frac{1}{\sum({}^{1}/_{\mathrm{Si}})}$$

The rating of water quality according to this WQI is given in Table 1.

Table 1. Rating of water quality according to WQI

WQI values	Rating of Water Quality	Grading
0-25	Excellent Quality	A
26-50	Good Water Quality	B
51-75	Poor Water Quality	C
76-100	Very Poor Water Quality	D
Above 100	Unsuitable for drinking purpose	E

Results and Discussion

In the WQI calculation high weight was assigned to oil and grease, TP, Nitrate-N, COD and pH (Table 2). The rating of water quality according to the WQI indicates excellent status (Grade A) in all wells in Vadamaradchi and poor to unsuitable for portability status in Chunnakam (Grade C, D or E). (Figure 2). The calculated WQI values in Vadamaradchi ranged from 7.79 to 16.91 while in Chunnakam ranged from 52.3 to 165.4 (Table 3).

High WQI in Chunnakam can be caused by elevated levels of oil and grease and nitrate. The water quality of Vadamaradchi shows the undisturbed aquifers by anthropogenic activities. The ground water quality may improve due to inflow of fresh water of good quality and dilution of contaminants during rainy season.

Table 2. Weight for each parameter

Chemical parameters	*Si*	*Wi*
pH	8.5	0.020
EC (µS/cm)	3500	$4.91*10^{-5}$
TS (mg/L)	2000	$8.61*10^{-5}$
TDS (mg/L)	500	$0.034*10^{-2}$
TH (mg/L,$CaCO_3$)	250	$0.069*10^{-2}$
COD (mg/L)	10	0.017
TP (mg/L)	2	0.086
Nitrate-N (mg/L)	11.2	0.015
Oil & grease (mg/L)	0.2	0.861

Table 3. Seasonal variation in WQI vales of sampled wells in Chunnakam (CW) and Vadamaradchi (VW)

Location	Well no	Average WQI	Grade	WQI-Dry season	Grade	WQI-Wet season	Grade
Polluted site (Chunnakam)	**CW1**	114.47	E	146.51	E	50.39	C
	CW2	138.38	E	168.61	E	77.92	D
	CW3	81.97	D	97.89	D	50.13	B
	CW4	52.13	C	63.50	C	29.40	B
	CW5	124.93	E	123.37	E	128.07	E
	CW6	119.85	E	167.19	E	25.15	A
	CW7	165.41	E	73.96	C	348.32	E
	CW8	62.22	C	67.56	C	51.54	C
	CW9	143.88	E	89.03	D	253.59	E
	CW10	104.48	E	119.69	E	74.06	C
Reference site (Vadamaradchi)	**VW1**	16.39	A	12.09	A	24.99	A
	VW2	12.29	A	12.44	A	11.99	A
	VW3	13.82	A	14.03	A	13.40	A
	VW4	10.10	A	11.85	A	6.60	A
	VW5	14.37	A	10.55	A	22.00	A
	VW6	13.50	A	12.91	A	14.67	A
	VW7	16.41	A	13.51	A	22.14	A
	VW8	9.89	A	10.91	A	7.86	A
	VW9	7.79	A	9.31	A	4.75	A
	VW10	16.91	A	14.94	A	20.87	A

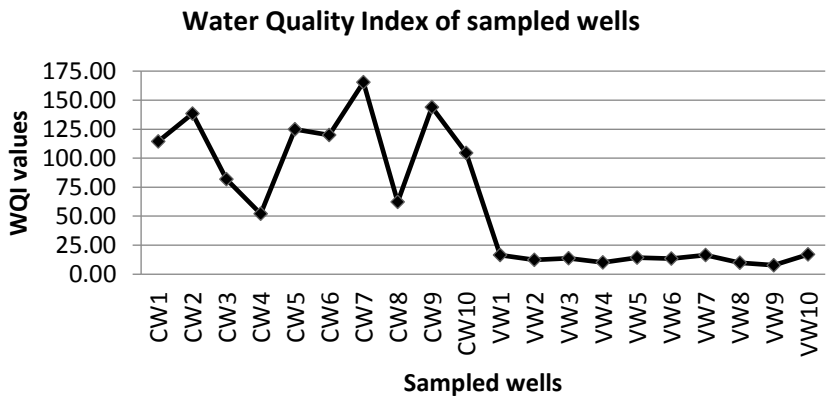


Figure 2. Values of WQI for all studied samples (CW- Chunnnakam); VW- Vadamaradchi)

Conclusion and Recommendation

Overall results conclude that domestic well water in Vadamaradchi aquifer is suitable for drinking water purpose while most of the wells in Chunnakam is not favorable for potability. Therefore, it is recommended that appropriate clean up and pollution prevention strategies need to be implemented in order to improve the ground water quality and to prevent further contamination of aquifer system in Chunnakam area in Jaffna peninsula.

References

[1] C.R. Panabokke and A.P.G.R.L. Perera Groundwater resources of Sri Lanka. Water Resources Board, Colombo, Sri Lanka. 2005, pp 1-29.

[2] F.B. Semiromi, A.H. Hassani, A. Torabian, A.R. Karbassi, F.H. Lotfi "Evolution of a new surface water quality index for Karoon catchment in Iran", *Water Science and Technology*, Vol 64 (2), pp 2483-2491, 2011.

[3] M. al-Hadithi "Application of water quality index to assess suitability of groundwater quality for drinking purposes in Ratmao–Pathri Rao watershed, Haridwar District, India", *American Journal of Scientific and Industrial Research*, vol 3 (6) pp 395-402, 2012.

[4] S. Harshan, M. Thushyanthy, J. Gunatilake, T. Srivaratharasan, K. Gunaalan. "Assessment of water quality index of groundwater quality in Chunnakam and Jaffna Town, Sri Lanka." *Vingnanam Journal of Science*. Vol 13 (1-2), pp 84 -91, 2017.

[5] S. Tyagi, B. Sharma, P. Singh, R. Dobhal "Water quality assessment in terms of water quality index", *American Journal of Water Resources*. Vol 1(3), pp 34-38, 2013.

FORMATION AND STABILITY OF JAROSITE ON MARS: AN ANALOG STUDY FROM THE EARTH

G.R.L. Kodikara* and L.J. McHenry

Department of Geosciences, University of Wisconsin-Milwaukee, USA.
**Corresponding author (email: gayantha@uwm.edu)*

Introduction

Jarosite, a ferric hydroxy sulfate mineral, generally forms in a slightly wet, oxidizing, acidic iron rich environments and rapidly decomposes to the ferric oxyhydroxides in more humid climates [1]. The Mossbauer instrument on the Mars Exploration Rover Opportunity identified the jarosite and hematite rich outcrops in Meridiani Planum at Eagle crater on Mars [2]. Later, Jarosite has been identified and mapped in the Mawrth Vallis region on Mars using Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) hyperspectral data [3]. Based on the corresponding mineral assemblages on the Earth, detection of jarosite on Mars may indicate the acidic and oxidizing aqueous environments [3].

The general formula for jarosite is $AB_3(XO_4)_2\ (OH)_6$. The A crystallographic site is occupied by K^+ in the case of endmember jarosite. Substitution of Na^+ for K^+ produces the natrojarosite end member. The crystallographic site B is occupied by Fe^{3+} in both end members. In the case of end member alunite and natroalunite, crystallographic site B is occupied by Al^{3+}. The tetrahedral site X is fully occupied by S (sulfur) in all end members [4]. Jarosite is highly sensitive to the changes of pH, temperature, oxidation condition and water abundance. Conventional thermodynamic stability models of jarosite show that Mars would need an acidic and water-limited environment to form and preserve jarosite over its history [1]. However, if jarosite is also found in terrestrial fresh water and saline-alkaline environments, jarosite may not be a good indicator of dominantly acidic conditions [3]. Therefore, the formation and stability of jarosite under different environmental conditions needs to be evaluated in detail to reconstruct past environments on Mars. McHenry et al. (2011) documented the presence of jarosite in altered tephra within the Pleistocene paleolake basin of Olduvai Gorge, Tanzania. Since the Olduvai paleolake was saline-alkaline rather than acidic, the presence of jarosite is unexpected [3]. Therefore, here we assess whether jarosite at Olduvai Gorge is formed by the oxidation of lacustrine pyrite under surface weathering conditions, or through some other alteration or mineral forming processes.

Materials and Methods

Geological framework

The Pleistocene Olduvai Gorge lacustrine basin is located in northern Tanzania between the Serengeti plains to the west and the Ngorongoro volcanic highlands (NVH) to the southeast (Fig. 1). The Olduvai formation (~ 100 m) is divided into a series of beds, including Beds I –IV, Masek, Ndutu, and Naisiusiu [3]. The Bed I, the oldest and thickest bed of the formation and Bed II, above Bed I, preserve saline-alkaline paleolake deposits formed between about 1.9 and 1.7 Ma [3]. Tuff IF, the uppermost tephra within Bed I, is trachytic to phonolitic and consists of multiple lava fragment-rich surge units, lapilli-dominated units, air fall units, and reworked units derived from the nearby NVH. The authigenic minerals in Tuff IF from sites across the paleolake form a classic bull's eye pattern, with clay-altered tephra in the distal lake margins, chabazite and phillipsite in the proximal margin, and phillipsite and K-feldspar in the intermittently dry lake and lake center [3].

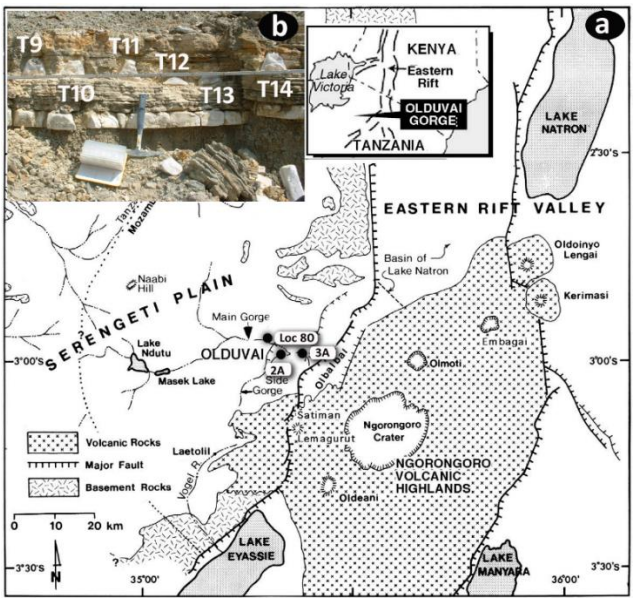


Figure 1. a) Regional map showing the location of Olduvai Gorge, Ngorongoro volcanic highlands and Serengeti plains. Modified after [3]. Sample locality 80 and the locations of two core samples (2A and 3A) are shown. b) Tuff IF at locality 80 with outcrop sample locations.

Method

Six samples of Tuff IF from two recent drill cores (Olduvai Gorge Drilling Project (OGDP) 2A and 3A) and six outcrop samples of the same tuff were analyzed. Six outcrop samples from Locality 80 were selected from a lateral transect of the finest tuff layer within Tuff IF, which had in past years yielded the highest abundances of jarosite, to determine whether jarosite was evenly distributed or "patchy" within this layer. Locality 80 contains of a series of altered, thin tuffs preserved within saline-alkaline lake sediments [3], of which Tuff IF is the thickest. Selected samples were cleaned to remove any crack fillings or roots. Cleaned samples were crushed and finely powdered for X-ray Diffraction (XRD) analysis. Samples were analyzed using a Bruker D8 Focus XRD (Cu Kα radiation, 2-60^0 2Θ, 0.2° step size, 1 second/step, scintillation detector). XRD patterns of the samples were compared against the ICDD (The International Centre for Diffraction Data) PDF-2 library using Bruker's EVA software package to identify the mineral constituents of the samples. The relative abundances of the minerals present in the sample were determined qualitatively based on their peak intensities.

Results and Discussion

Analyzed XRD results are shown in Table 1. Most of the surface outcrop samples contain identifiable jarosite peaks above background, while none of the core samples indicate jarosite. One core sample shows a relatively high amount of pyrite. Outcrop samples do not appear to contain pyrite except for very low amounts in sample LM-12-T14. Phillipsite, sanidine and smectite are identified in all the samples.

The first step in the overall process of jarosite formation at Olduvai was most likely the formation of pyrite in an anoxic saline-alkaline lake environment, likely by bacterial reduction of sulfate [5]. The presence of pyrite in the lake bed is confirmed by the physical descriptions of the cores, and the presence of pyrite in Tuff IF is confirmed by XRD of core samples 3A-24Y-1-53-55 and 2A-28Y-1-80-83. More recently, pyrite oxidized in to Fe^{2+} and SO_4^{2-} via oxidation upon exposure to surface conditions in outcrop. McHenry et al. (2011) found that K and Na are abundant in altered Tuff IF, in the original phonolotic volcanic glass (where preserved), in anorthoclase, and in secondary minerals including the zeolite phillispsite and authigenic K-feldspar. The concentration of K^+ relative to Na^+ (as observed in zeolites) increased with proximity to the lake center [3]. High concentrations of available K^+ governed the formation of jarosite instead of a natrojarosite endmember. Presence of analcime and phillipsite also depicts the alkaline nature (high pH) of the Olduvai Gorge, Tanzania [3].

Table1. Authigenic mineral assemblages of each sample identified by XRD

	Outcrop samples						Core samples					
Mineral Name	LM-12-T9	LM-12-T10	LM-12-T11	LM-12-T12	LM-12-T13	LM-12-T14	2A-28Y-1-80-83	2A-28Y-1-85-86	2A-28Y-2-10-12	3A-24Y-1-46-48	3A-24Y-1-53-55	3A-24Y-1-75-77
Analcime	-	-	-	-	-	-	xx	-	-	-	-	-
Ferrihydrite	-	-	xx	-	-	-	-	-	-	-	-	-
Goethite	xx	x	xx	-	xx	x	-	-	x	-	-	xx
Jarosite	x	-	xx	xx	xx	xx	-	-	-	-	-	-
Smectite	x	xx	x	xx	xx	x	xx	xx	xx	x	x	xx
Orthoclase	-	-	-	-	-	xx	-	xx	-	-	xxx	-
Pyrite	-	-	-	-	-	x	x	-	-	-	xxx	-
Phillipsite-K	xxx x	xxx x	xxx x	xxx x	xxx x	xxx x	xxx x	xxx x	xxx	xxx x	x	xxx
Sanidine	xx	xxx	xx	xxx	xxx	x	xx	xxx x	xxx x	xxx	xxx x	xxx x

xxxx = Abundant, xxx = Common, xx = Rare to common, x = Rare, - = Absent (Abundances estimated qualitatively based on relative peak heights).

Conclusions and Recommendations

XRD analysis along with redox reaction studies suggested that, the jarosite could be formed due to the oxidation of sedimentary pyrite formed by the sulfate reduction of organic matter. H^+ released during the oxidation of pyrite, provides a local and temporary acidic environment for jarosite formation. Availability of higher concentration of K^+ than Na^+ at the lake center governed the formation of jarosite instead of a natrojarosite endmember. Presence of analcime and phillipsite in the same mineral assemblage depicts the alkaline nature (high pH) of the Olduvai Gorge, Tanzania. Most importantly, this study suggested that, jarosite could stable even in basic conditions (high pH), therefore only based on the presence of jarosite, it is difficult to conclude the paleo environment on Mars as an acidic environment.

References

[1] Maden, M.E.E., Bodnar, R.J. and Rimstidt, J.D., "Jarosite as an indicator of water limited chemical weathering on Mars." nature, Vol. 431: 821-823, 2004.

[2] Klingelhofer, G. et al., "Jarosite and Hematite at Meridiani Planum from Opportunity's Mossbauer Spectrometer." Science, Vol. 306: 1740-1745, 2004.

[3] McHenry, L.J., Chevrier, V. and Schröder, C., "Jarosite in a Pleistocene East African saline-alkaline paleolacustrine deposit: Implications for Mars aqueous geochemistry." Journal of Geophysical Research, Vol. 116(E04002): doi:10.1029/2010JE003680, 2011.

[4] Papike, J.J., Karner, J.M. and Shearer, C.K., "Comparative planetary mineralogy: Implications of martian and terrestrial jarosite. A crystal chemical perspective." Geochemica et Cosmochimica Acta, 70: 1309-1321, 2006.

[5] Berner, R.A., "Sedimentary pyrite formation: An update." Geochemica et Cosmochimica Acta, 48: 605-615, 1984.

IDENTIFICATION OF NANTHIKADAL DRAINAGE BASIN USING DIGITAL ELEVATION MODEL

S. Muraleetharan*[1] and P. Surenthar[2]

[1]*Central Environmental Authority, Sri Lanka*
[2]*Department of Fisheries, University of Jaffna, Sri Lanka*
**Corresponding author (email: muraly_3@yahoo.com)*

Introduction

Coastal lagoons are defined as shallow water bodies separated from the ocean by a barrier, connected to it at least temporarily by one or more restricted inlets and usually oriented parallel to the shore. They trap inorganic sediment and organic matter, and thus serve as material sinks or material filters. They often exhibit very high primary and secondary production rates and are valuable for fisheries, aquaculture and sometimes for salt extraction.

Sri Lanka has about 80 lagoons located along its 1,338 km coastline [1]. These natural coastal resources are national assets which play a vital role in the island's ecology and the nation's economy. Lagoons have many values. The use values (Fish, Shrimp, Fuel wood, Salt, Fodder, Ecotourism, Anchorage, Recreation etc.) and non-use values (Habitat preservation, Mangroves, Sea-grass beds, Biodiversity, Ecosystem linkages etc.) contribute significantly to human well-being of coastal communities, constituting about 12% of Sri Lanka's population. This indicates that 1 in 8 Sri Lankans live in locations connected directly or indirectly with lagoons [2].

Nanthikadal is one of the largest lagoons in the northern coastal zone [1]. It is linked to the sea by a narrow channel to the east, near Mullaitivu town and surrounded by rice paddies and coconut plantations. Lagoon fishing is popular among the fishing community in Mullaitivu. Lagoon prawns are in high taste and demand. Among the many values generated by Nanthikadal lagoon, the value of the fish and shrimp catches is predominant. The existence of fishery is closely related to lagoon productivity and ecology which is balanced by different factors. One such factor is drainage basin. Drainage basin is a region of land where water from rain drains downhill into a body of water, such as a river, lake or lagoon. As water flows over the ground and along rivers it can pick up nutrients, sediment, and pollutants. Like the water, they get transported towards the lagoon, and can affect, nutrient dynamics and primary productivity which intern affect the fishery of Nanthikdal lagoon.

Therefore, identifying the drainage basin is important not only for planning and implementation of water resource management, but also for the survival of the fishing community living around. Due to the two decades of civil war, there are

no data on Nanthikadal lagoon drainage basin. Therefore the objective of this study is to identify the lagoon drainage basin.

The traditional method to identify drainage basin is from topographic map that contains drawing lines to connecting elevation points and contour lines. It is laborious and time consuming job and with the advance in geographical information system (GIS), it was widely replaced by the tools developed to identify hydrologic basins using digital elevation models. Using digital topographic data (Aster GDEM) and GIS technology (ESRI's ArcGIS 10.3) the basin were delineate manually on screen [3, 4].

Methodology

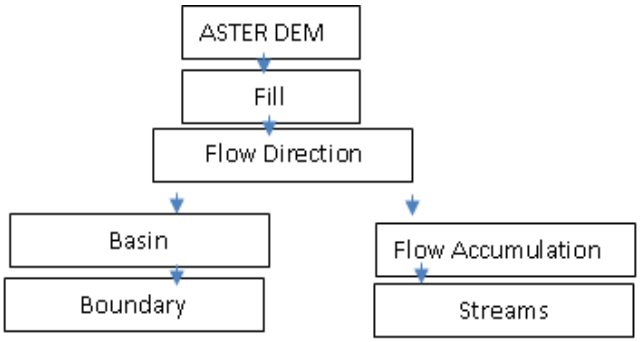


Figure 1. Flow chart of methodology

Figure 1 shows the flowchart of methodology followed in this research. Advanced space borne thermal Emission Reflection Radiometer Global DEM (ASTER DEM) data is used to identify the drainage basin. (https://earthexplorer.usgs.gov/). Then the desired area is clipped to DEM voids. Calculation of flow direction and flow accumulation is done using HYDROLOGY TOOLS available under the extension SPATIAL ANALYSTS TOOLS. A stream threshold of 60000 applied to calculate the dominant streams in the study area.

Study Site

Nanthikadal Lagoon is located in the North east area of Sri Lanka. The GPS location of the lagoon is 9°17'0"N 80°46'0"E. This water body at present is a saline coastal lagoon with the surface area of 46.7 km^2. The lagoon mouth keeps closed for the most of the year and it's intermittently opened naturally during rainy season or artificially by Lagoon management committee.

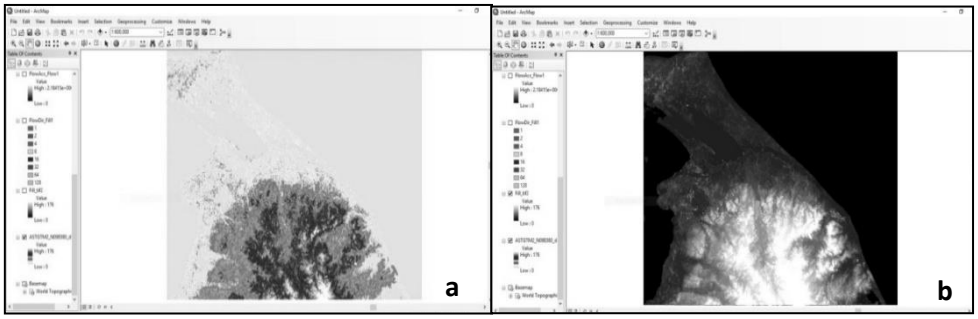


Figure 2. (a) DEM obtained from Earthexplorer, shows the digital elevation of the northern part of Sri Lanka. The elevation ranges from 0 to 176. **(b)** Filled DEM

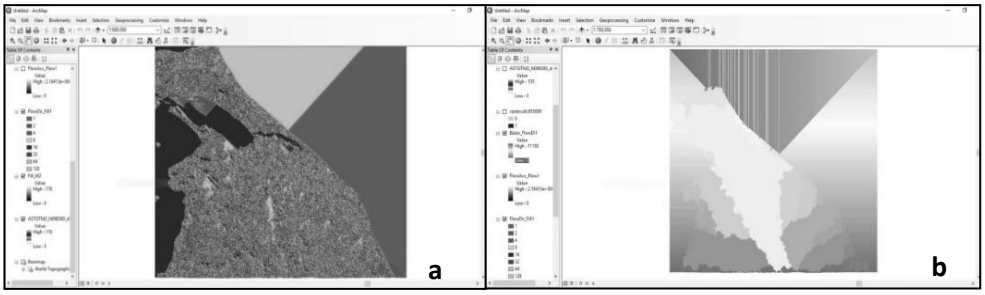


Figure 3: (a) Flow Direction **(b)** Drainage Basin

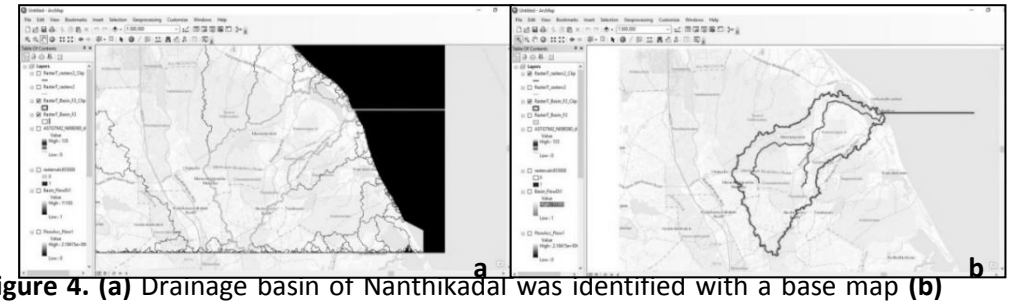


Figure 4. (a) Drainage basin of Nanthikadal was identified with a base map **(b)** Stream network of the corresponding basin

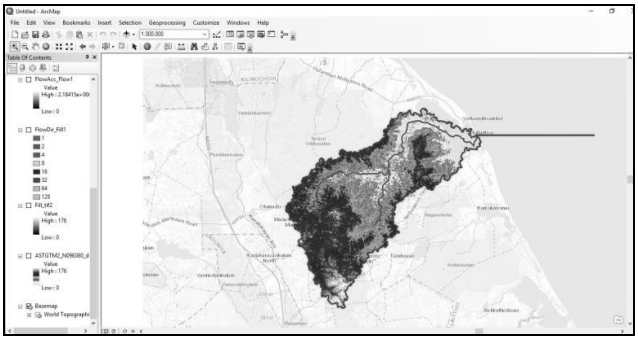


Figure 5. Stream network of the corresponding basin

Results and Discussion

It is to be emphasized that a vast potential for the development of fisheries exists in Nanthikadal lagoon. While civil war must have had a depressing effect on new entrants to fisheries it has also led to a very low rate of acquisition of new crafts and gear. In fact, many people refrained from fishing during the civil war. Now that the war is over, people have commenced fishing in lagoons. It became as a major source of their income.

After the civil war, people have been busy in clearing land for farming, sand and gravel mining, building towns and changing drainage basin of the Nanthikadal lagoon which was in equilibrium for thousands of years. There should be a sustainable development plan which does not affect the ecology and productivity of the lagoon. Although the existence of fishery is closely related to lagoon productivity and ecology, fisheries do not exist at all lagoons in Sri Lanka. Of the 80 lagoons in Sri Lanka fisheries exist only in about half of them. Therefore, it is vital to implement a sustainable development that is not affecting the fisheries of Nanthikadal. One clear example of mismanagement is the construction of causeway across the lagoon which resulted landsides of the lagoons to be "fresher" than "brine," leading to a decline in shrimp production.

It is important of a sustainable development plan and gaining knowledge of the lagoon ecology in order to protect it. This research is a small step towards this direction.

The drainage basin of Nanthikadal was identified with the help of base map of Sri Lanka. The stream network generated by the ArcGIS is also aligning with the natural river network (Per Aru) that present in the corresponding basin. However, there are small deviations resulted due to the spatial resolution of data.

Conclusion

Careful management of identifying and allocating water resource is important to preserve water for the next generation. Accurate delineation of drainage basin plays an important role in sustainable development and management of the lagoon. In this research, the drainage basin of Nanthikadal is identified. This can be useful in modern water resource management and provide support to policy makers in their decision making. In addition, it is also useful to calculate the basins hydrologic and topographic features for developing a rainfall – runoff model.

References

[1] EIL Silva, J Katupotha, O Amarasinghe, H Manthrithilake, R Ariyaratna. Lagoons of Sri Lanka: from the origins to the present. IWMI; 2013.

[2] Ministry of Fisheries & Aquatic Resources Development Sri Lanka and IUCN, International Union for Conservation of Nature & Natural Resources (Sri

Lanka). Consultative Workshop on Sustainable Management of Lagoons. 23rd June, 2016.

[3] RM Singh, SP Maurya. River network identification using Remote Sensing and GIS. InEngineering and Systems (SCES), 2014 Students Conference on 2014 May 28 (pp. 1-6). IEEE.

[4] US Visharolia, NJ Shrimali, I Prakash. Watershed delineation of purna river using geographical information system(GIS). International Journal of Advance Engineering and Research Development, 4(5), 690-695, 2017.

PREPARATION AND CHARACTERIZATION OF MUNICIPAL SOLID WASTE BASED CLAY BIOCHAR COMPOSITE FOR QUINOLONE ANTIBIOTIC REMOVAL

A. Ashiq, N. Adassooriya, A.U. Rajapaksha, M. Vithanage*

Ecosphere Resilience Research Centre, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka
**Corresponding author (email: meththika@sjp.ac.lk)*

Introduction

Contamination of water bodies by antibiotics has become an environmental concern in the recent decades and hence they have been considered as emerging contaminants. These antibiotics are used both in human and in veterinary medicine, which make them quite abundant in the environment. They could affect the aquatic organisms through developing bacterial resistances and releases toxic chemicals. Ciprofloxacin (CPX), which belongs to the fluoroquinolone group of antibiotics, is commonly detected from livestock, soil and water due to its stable nature and compatibility to a wide range of bacteria. It has been proven to be highly genotoxic in nature damaging ecosystems. Thus, removal of CPX from aqueous systems has become an urgent need [1].

Over the past years, there have been approaches to improve bio-sorbents from different biomass sources including municipal solid wastes as a route for sustainable production and consumption. Ahmad et al., (2014) reviews the usage of biochar for both organic and inorganic sorbates [2]. Often municipal solid waste is dumped predominantly into open dumps which is typically a low cost approach and hazardous to the environment at the same time; its usability as a sorbent by the conversion to biochar has been proven as potential in many of the recent studies [3]. In order to improve biochar's capacity for removal of antibiotics, composites have been made with different clay materials [4]. Therefore, the objectives of this study were to assess the plausibility of clay-aided biochar composite to improve the sorption of CPX and to examine their application as an antibiotic sorbent through morphologies investigated from characterization techniques applied.

Materials and methods

Preparation of biochar

Municipal solid waste (MSW) obtained from Gohagoda dumpsite, Kandy, was used for the production of biochar. Partially dried MSW was pyrolyzed at 450 °C in the muffle furnace with 15 °C/min increasing rate of temperature and 30

minutes holding time. The pyrolyzed biomass is then being immediately quenched to have the pores activated rather than being converted into ashes with air to obtain the pristine MSW biochar (MSW-BC).

A suspension was created using 50 g of bentonite and 2 L of deionized water and sonicated for 30 min. The segregated organic part of MSW (250 g) was then added into a clay slurry suspension and then shaken for 2 hours. Once the MSW has been properly treated with bentonite, it is then oven dried at 80 °C for overnight. The prepared MSW treated clay is being converted to biochar via slow pyrolysis at 450 °C in a muffle furnace as the procedure repeats the same as the preparation of MSW-BC.

Similar procedures were followed to prepare biochar-montmorillonite composite except that the MSW-BC were added into the montmorillonite (MMT) clay suspension previously made with 5 g clay suspension then oven dried overnight.

All of the three sorbents: municipal solid waste biochar (BC), biochar-bentonite composite (BC-BEN) and biochar-montmorillonite composite (BC-MMT) were treated with ciprofloxacin (CPX) 100 mg L^{-1} in deionized water. At each equilibrated stage, an aliquot from each batch was filtered through a 0.45 μm syringe filter and then the final solutions were analyzed using UV-Visible spectrophotometer (Shimadzu UV160A) at 280 nm wavelength.

Characterization of biochar

Fourier transform infrared spectroscopy (FT-IR) was used to determine the surface functional groups with the recording range from 4000 to 400 cm^{-1}. The FT-IR (Nicolet 6700 FT-IR spectrophotometer) spectra were analyzed using OMNIC version 7.3 software. Powder X-ray diffraction (PXRD) patterns of biochar and composites were obtained on a X-ray diffractometer (Rigaku, Ultima IV, Japan) with Cu Kα radiations and wavelength of 1.54056 Å. The PXRD traces of all working samples were recorded with over a 2 theta range of 3–60^{o} with a step size of 0.02^{o} and a step time of 1 second. The samples were freeze dried, crushed, sieved (100 mesh) and a thin film of the powder was before placing in to the PXRD.

Results and Discussion

PXRD Characterization

Powder X-ray diffraction traces for MSW-BC, pristine bentonite (BEN), BC-BEN, BC-BEN treated with CPX as shown in Figure 7 (a); the first peak indicates the basal reflection (001) plane of the pattern. The spectra for biochar show a

noticeable peak at 2θ of 24^{o} which reveals the presence of quartz. There is a decrease in the intensity shown in the peaks after the composites are being prepared and treated with CPX. This accounts for the internal structure and morphological change in the clay so produced after it is been coated on the MSW-BC mixture.

There is a subtle shift in the basal plane noticed when the BC is being modified with the clay and when treated with the antibiotic. The interlayer spacing clearly indicates the shift of the pattern to the left after CPX has been incorporated and it can attribute to its contribution within the lattice structure of the BC-clay composite.

The basal reflection of the composites showed a shift to the right in the peak compared to the pure Montmorillonite and CPX treated composite. Apart from that, montmorillonite BC composite created not much of a deflection when treated with CPX.

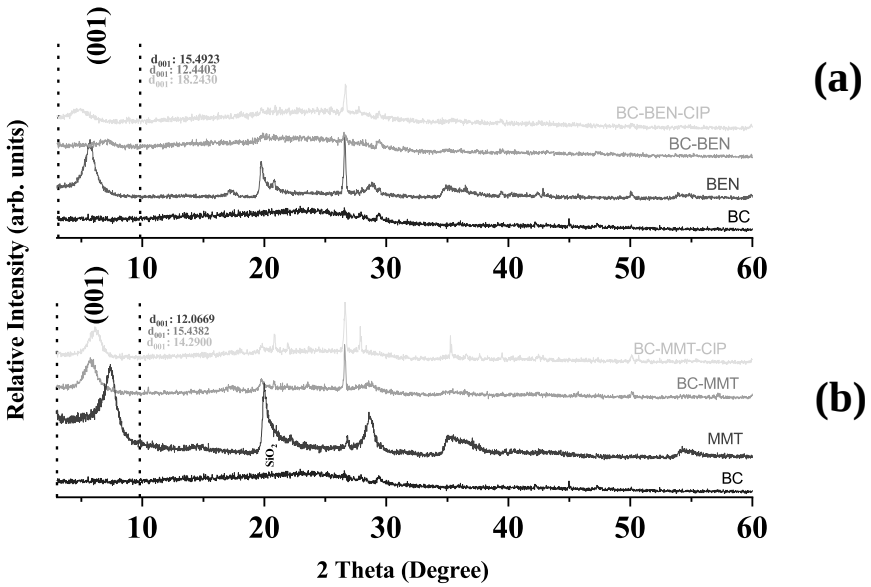


Figure 7. PXRD patterns of (a) biochar-bentonite composite and their parent material (b) biochar-montmorillonite composite and their parent material

FT-IR Characterization

The types of the functional groups are identified in the molecules in FT-IR spectra (Figure 8). Spectrum range 3000- 4000 cm^{-1} region showed the stretching of the hydroxyl groups that was initially present in the bentonite. The disappearance of hydroxyl group is accounted due to the biochar incorporation [5]. There is a presence of a C=O group that was ruled out for the MMT at 1632 cm^{-1} wavenumber which showed disappearance when the composites prepared as well. The Si-O bond that was found in bentonite at 1006 cm^{-1} showed a drastic decrease in the intensity for the composites and the CPX treated composites.

Conclusions and Recommendations

The plausibility for the removal of quinolone-based antibiotic- ciprofloxacin was investigated through the spectral patterns. Overall, the composites showed a significant change in the crystallinity and functional groups compared with the pristine material. The FT-IR spectral analyses of the composites before and after CPX treatment confirm the adsorption process and the mechanism can be predicted only by investigating further adsorption series experiments. The PXRD patterns confirm the intercalation of the CPX molecules in to the crystalline structures of the composites through the obtained basal reflections. The interactions of CPX with the composites should be further investigated and needs to consider the amphoteric nature CPX molecule in different solution, to further retain CPX on the composite for the efficient removal.

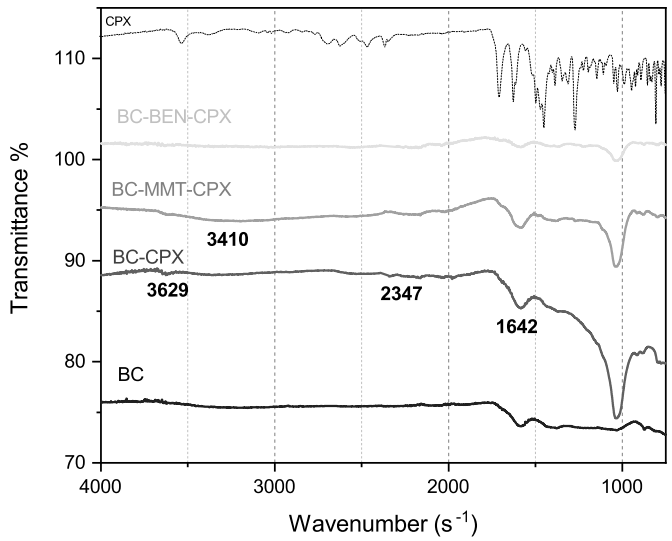


Figure 8. FT-IR spectra of pristine MSW-BC and treated composite with CPX

Acknowledgements

Financial support from the grant ASP/01/RE/SCI/2017/83 from the Research Council, and the analytical support from the Instrument Center, Faculty of Applied Sciences, University of Sri Jayewardenepura are acknowledged.

References

[1] J. Li *et al.*, "Study of ciprofloxacin removal by biochar obtained from used tea leaves," *J. Environ. Sci. (China)*, pp. 1–11, 2018.

[2] M. Ahmad *et al.*, "Biochar as a sorbent for contaminant management in soil and water: A review," *Chemosphere*, vol. 99. pp. 19–23, 2014.

[3] V. Gunarathne, A. Ashiq, and M. P. Ginige, "Green Adsorbents for Pollutant Removal," vol. 19, 2018.

[4] W. Duan, N. Wang, W. Xiao, Y. Zhao, and Y. Zheng, "Ciprofloxacin adsorption onto different micro-structured tourmaline, halloysite and biotite," *J. Mol. Liq.*, p. #pagerange#, 2018.

[5] Q. Wu, Z. Li, H. Hong, K. Yin, and L. Tie, "Adsorption and intercalation of ciprofloxacin on montmorillonite," *Appl. Clay Sci.*, vol. 50, no. 2, pp. 204–211, 2010.

HEAVY METAL FRACTIONATION AND ACID LEACHING FROM ELECTRICAL AND BATTERY INDUSTRIAL SLUDGE

V. Gunarathne[1], A.U. Rajapaksha*[1], N. Adassooriya[1], A. Cooray[2], M. Vithanage[1]

[1]Ecosphere Resilience Research Centre, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka
[2]Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka
**Corresponding author (email: anurajapaksha@sjp.ac.lk)*

Introduction

Rapid development of industries caused the generation of large quantities of industrial wastes. Among different types of industrial wastes, industrial sludge has been considered as one of the problematic waste because of the elevated content of various toxic metals. Industrial activities such as electroplating and battery manufacturing generate massive quantities of sludge which are rich in heavy metals such as Pb, Cd, Zn, Ni and Cu [1]. Battery and electrical appliance industries in Sri Lanka generate trace metal rich sludge and limited treatment techniques result direct discharging of sludge to the environment [2]. Release of toxic metals into the natural environment may create numerous environmental and human health related consequences. Therefore, treatment of industrial sludge using proper treatment method is mandatory and helpful for resource recovery.

Thickening, biological and chemical stabilization, conditioning and dehydration are the widely used conventional treatment techniques for industrial sludge. However, acid leaching technique has been well recognized as an effective treatment method for various kinds of industrial sludge as well as to recover precious metals form the sludge. Prior to the treatment process, an understanding of the properties of sludge type is essential because, diverse sludge types may influence the treatment process. Further, the types of the metals present in the sludge and their fractionation among different phases of the sludge might be important determinants to adopt the most suitable acid type for leaching. Therefore, the aim of this study is to characterize two industrial sludge types in order to facilitate acid leaching process to recover valuable metals.

Materials and Methods

Two industrial sludge types from battery manufacturing industry and electronic industry were air dried, crushed and mechanically sieved to take < 1 mm fraction for further analysis. Chemical properties of sludge including; pH, electrical conductivity (EC), available N and P, percentage total organic carbon (TOC) and cation exchange capacity (CEC) were analyzed. The pH and EC of two sludge types were measured using the solution of 1:10 ratio of sludge and deionized water.

Available N, P and TOC were determined using standard colorimetric methods [3]. Ammonium acetate extraction procedure was followed to determine CEC. Diethylene triamine pentaacetic acid (DTPA) extraction procedure was used to quantify bioavailable fraction of heavy metals. Sequential extraction procedure was carried out to determine the metal bound phases namely; Exchangeable, Bound to carbonates, Bound to Fe-Mn oxide, Bound to organic matter, and Residual fractions [4].

Results and Discussion

The properties of industrial sludge types primarily depend upon the nature of raw materials used and the manufacturing process. The two industrial sludge types examined differ each other by their basic chemical properties (Table 1). The sludge resulted through the battery manufacturing process (Battery sludge) contained higher available N and CEC values than the sludge from electronic product industry (Electronic sludge) whilst electronic sludge contains higher EC, available P and TOC over battery sludge. However, the pH is neutral for both sludge types, might be due to the pH neutralization treatment which was done before releasing of sludge into the natural environment.

Table 1. Chemical properties of two industrial sludge types

Sludge type	pH (1:10)	EC (mS/cm)	Available N (mg/kg)	Available P (mg/kg)	TOC %	CEC ($cmol_c/kg$)
Battery sludge	7.27	10.78	230.01	1.35	4.79	287.85
Electronic sludge	7.92	461.33	1.91	709.07	10.24	19.55

EC= Electrical conductivity, TOC= Total organic carbon and CEC= Cation exchange capacity

It is well known that the DTPA extraction procedure gives reliable results to evaluate bioavailable trace metals in soils and sediments [5]. The concentration of Pb, Cu, Zn, Ni and Cd in DTPA extracted fraction was given in Table 2. Battery sludge contained an elevated bioavailable concentration of Pb (296.61±20.31 mg/kg) whilst electronic sludge comprised with high concentrations of Cu (298.65±3.28 mg/kg). Both sludge types contained comparatively similar concentrations of Zn however, reported bioavailable concentrations of Ni and Cd were very low (Table 2).

Table 2. Bioavailable concentrations of metals present in each sludge type

Sludge type	Bioavailable concentration (mg/kg)				
	Pb	**Cu**	**Zn**	**Ni**	**Cd**
Battery sludge	296.61 (20.31)	12.10 (0.78)	65.24 (4.10)	4.00 (0.32)	0.12 (0.00)
Electronic sludge	2.33 (0.03)	298.65 (3.28)	51.75 (0.41)	1.75 (0.05)	ND

**Values given in parenthesis are standard deviations of triplicates.*

The chemical form and the bonding structure of metal ions in solid phase have great impact on leaching process. Sequential extraction procedure gives useful evidences about the chemical form of metals among different fractions of sludge (i.e. Exchangeable, Bound to carbonates, Bound to Fe-Mn oxide, Bound to organic matter, and Residual). Lead in the battery sludge is mostly bound to Fe-Mn oxide and carbonate fractions (59.0 and 32.3 % respectively) (Figure 1). Similarly, Cu in electronic sludge distributed among carbonate and Fe-Mn oxide fractions (65.0 and 25.8 % respectively). According to the results, Pb in battery sludge and Cu in electronic sludge are predominantly distributed in carbonate and Fe-Mn oxide fractions. High amount of metals present in carbonate bound fraction could be a promising sign in order to utilize acid leaching for recovery of metals from sludge.

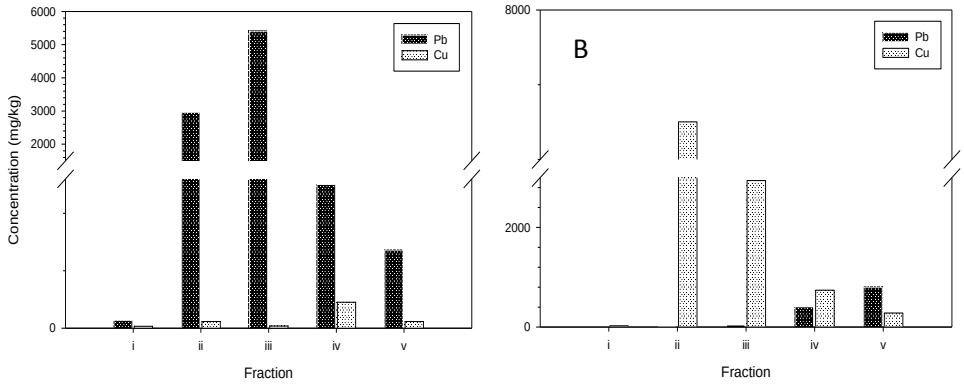


Figure 1. Distribution of Pb and Cu among; i) Exchangeable, ii) Bound to carbonates, iii) Bound to Fe-Mn oxide, iv) Bound to organic matter, and v) Residual fractions of sludge. (A – Battery sludge, B – Electronic sludge).

Conclusions and Recommendations

Both industrial sludge types examined in this study contain Pb, Cu, Zn and Ni in bioavailable fraction. However, Pb is the most abundant bioavailable metal in battery sludge whilst high bioavailable concentration of Cu was found in electronic sludge. Both Pb and Cu were mainly distributed among carbonate and Fe-Mn oxide fractions of sludge. Future studies are in line to find out suitable acid

type, concentration and solid: liquid ratio for effective recovery of metals from each sludge type.

Acknowledgement

Financial assistance by Research Council under the research grant No ASP/01/RE/SCI/2017/82 and the analytical support from the Instrument Center, Faculty of Applied Sciences and University of Sri Jayewardenepura are acknowledged.

References

[1] C. Li *et al.*, "Multiple heavy metals extraction and recovery from hazardous electroplating sludge waste via ultrasonically enhanced two-stage acid leaching," *Journal of Hazardous materials,* vol. 178, no. 1-3, pp. 823-833, 2010.

[2] K. Anjithan, B. Athapattu, N. Ratnayake, and L. Udamulla, "Sludge of Water Treatment Works: Are Disposal Practices Sustainable," 2015.

[3] J. M. Anderson and J. Ingram, *Tropical soil biology and fertility*. CAB international Wallingford, 1989.

[4] A. Tessier, P. G. Campbell, and M. Bisson, "Sequential extraction procedure for the speciation of particulate trace metals," *Analytical chemistry,* vol. 51, no. 7, pp. 844-851, 1979.

[5] M. Kashem, B. Singh, T. Kondo, S. I. Huq, and S. Kawai, "Comparison of extractability of Cd, Cu, Pb and Zn with sequential extraction in contaminated and non-contaminated soils," *International Journal of Environmental Science & Technology,* vol. 4, no. 2, pp. 169-176, 2007.

A NEWLY ISOLATED AEROBIC BACTERIAL STRAIN (*Paenebacillus polymyxa) T*OWARDS REMEDIATION OF NITRATE POLLUTION OF WATER

A. Thurairajah[1, 2]*, N. Gnanavelrajah[1], R.R. Ratnayake[2]

[1]*Faculty of Agriculture, University of Jaffna, Sri Lanka*
[2]*National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka*
**Corresponding author (email: abhiramyt@univ.jfn.ac.lk)*

Introduction

Nitrate contamination of ground water has become a serious issue all over the world. Excessive consumption of nitrate can cause health effect to human and animals; specially associated to methemoglobinemia (blue baby syndrome) in infants and gastrointestinal cancer in adults (Ren et al., 2018). Bacterial denitrification is being considered to be the promising approach for reducing nitrate from contaminated water. Biological denitrification is the anaerobic processes of reduction of oxidized nitrogen compounds through the sequential activity of microbial reductase enzymes to gaseous nitrogen. Four enzymes, such as nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase are responsible for complete reduction of nitrate ion to di nitrogen gas. There are varieties of incomplete denitrification pathways also exist: Few denitrifying bacteria reduce both nitrate and nitrite; others reduce only nitrite, Few produce only dinitrogen; others produce a mixture of dinitrogen and nitrous oxide; others produce only nitrous oxide (Carlson and Ingraham, 1983). Biological deitrification is the most important and widely used method to treat nitrate wastes as it enables the conversion of nitrogen compounds in to harmless di nitrogen gas. Although nitrate reduction activity is exhibited by diversity of microbial genera, with a range of heterotrophic and autotrophic metabolism, the aerobic nitrate reducers belongs to a very restricted group. Aerobic denitrification is attracted a lots of attention due to its easier operation and higher nitrate reduction efficiency than anaerobic denitrification (Wu et al., 2013). Most predominant denitifying bacteria in our environment which has been frequently reported belongs to the genus *Pseudomonas.* There are reports on aerobic denitrifying species isolated from environmental samples such as; ponds, canals, soils and activated sludge (Wu et al., 2013). Consequently, the aim of this study was to investigate the efficiency of bacteriological removal of nitrate from nitrate rich medium and water by newly isolated strain in laboratory condition. The effect of two carbon sources glucose and starch in three different percentages were investigated using mineral salt medium containing KNO_3 to select the most effective carbon source.

Materials and Methods

Isolation and Primary Screening

Microorganism used in this study was isolated from paddy field in Jaffna District, Sri Lanka. Modified seed medium (SM) and Bromothymol Blue Medium (BTB) was used to screen the denitrifier in aerobic condition. The modified SM consisted of (g/l): peptone 5.0, NaCl 5.0, KNO_3 1.0, Glucose 1.0 at pH 7.0. The modified BTB medium included (0.1% L-asparagine, 0.1% KNO_3, 0.1% KH_2PO_4, 0.005% $FeCl_2.6H_2O$, 0.02% $CaCl_2.2H_2O$, 0.1% $MgSO_4.7H_2O$, 1 ml of BTB liter1 [1% in ethanol], 2% agar, 0.5% glucose; pH 7.0) (Takaya et al., 2003). Nutrient broth with KNO_3 was used to check denitrification activity as initial screening (Blaszczyk, 1993).

Identification of Isolated Bacterium

Various standard physiological and biochemical characteristics were inspected. Pure culture was submitted to the gene tech, Sri Lanka for the 16S rRNA homology analysis. Sequences were analyzed using Bio Edit sequence alignment editor.

Nitrate Reduction in Synthetic Medium and water

Nitrate removal activity of best isolate of A19 (*Paenebacillus polymyxa*) was first evaluated in a synthetic Mineral salt medium (MSM) consisted of (potassium dihydrogen phosphate, 0.1 g l^{-1}; dipotassium hydrogen phosphate, 1 g l^{-1}; ammonium chloride, 0.5 g l^{-1}; calcium chloride, 0.005 g l^{-1}; magnesium sulphate, 0.1 g l^{-1}; sodium silicate, 0.05 g l^{-1}; pH 7.2) with glucose and starch carbon sources with three different percentage (0.25%, 0.5% and 1%) (Ayyasamy et al., 2007). Medium with glucose and starch was sterilized at 110^{o}C, 15 psi for 15 minutes and 121^{o}C, 15 psi for 20 minutes respectively. Similarly, the cell suspension was inoculated to 50 ml of filter sterilized (0.45 µm syringe filter) groundwater sample collected from the Jaffna district, containing 57.37 mg l^{-1} nitrate, which was supplemented with 0. 5 % starch. It was incubated at 30^{o}C temperature for 72h at 120 rpm in shaker incubator. At 12 h interval, the utilization of nitrate was determined.

Analytical Methods

Nitrate determination of the bacterial inoculated sample was conducted with use of Spectrophotometer (410 nm) via reaction with salicylic acid and sodium hydroxide. Nitrite was measured spectrophotometrycally (520 nm) through the reaction with sulfanilic acid and N, N –dimethyl -1- naphthylamine. Data was analyzed using General Linear Model in Analysis of Variance (ANOVA-GLM). All comparisons were done using Minitab 16.

Results

Isolation and Identification of Aerobic Denitrifying Bacterium

Bacteria capable of reducing nitrate to nitrogen gas were isolated from paddy soil sample. Several strains were isolated from the first screening based on blue colony formation on the BTB agar plates due to an increase of pH on the medium (Wu etn al., 2013). After rescreening, the nitrate removal efficiency was examined in nutrient broth with nitrate under aerobic conditions. One strain which we denoted as A19 had high nitrate removal rate of 98.18% was selected for further screening using synthetic medium (MSM) with nitrate supplement and Glucose and Starch sole carbon sources. Eventually, cells of strain A19 grown well in the medium contain starch and glucose as carbon sources. The isolate was identified as *Paeneibacillus polymyxa* based on its physiological and biochemical characteristics and the results of 16S rRNA gene homology analysis. Morphological and biochemical characteristics of *Paenebacillus polymyxa* is given in Table 1.

Table 1. Taxonomical characteristics of isolated aerobic denitrifying bacteria

Identification test	Result
Physiological test	
Motility test	Motile
Morphological test	
Colony morphology	Round
Gram stain	Positive
Shape under microscope	Short rod
Margin	Regular
Biochemical test	
Oxidase test	Positive
Catalase test	Positive
Nitrate reduction test	Positive
Growth on MacConkey agar	Negative

Aerobic nitrate removal of *Paenebacillus polymyxa* in Synthetic medium (Mineral Salt Medium (MSM) and in water

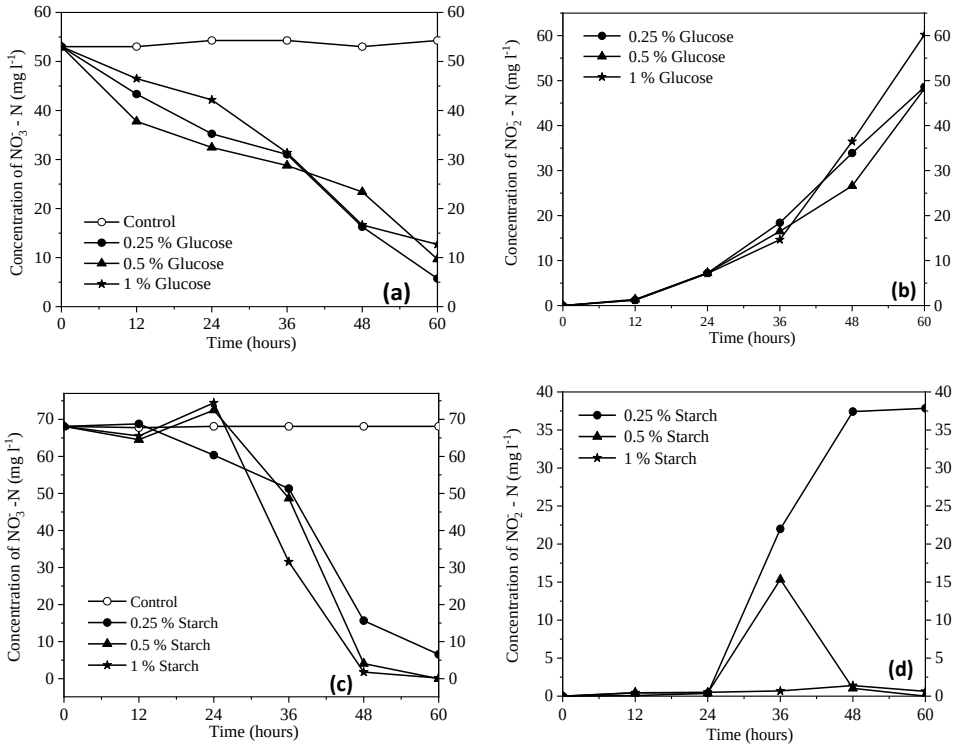


Figure 9: Concentration of nitrate and nitrite on mineral salt medium treated with *Paenibacillu polymyxa* with different % of glucose and Starch. (a) Concentration of nitrate with glucose, (b) Concentration of nitrite with glucose, (c) Concentration of nitrate with starch, (d) Concentration of nitrite with starch.

Since, the strain *Paenebacillus polymyxa* is a heterotrophic organism capable to degrade starch and glucose. For all concentrations nitrate was decreased gradually with time, meanwhile nitrite was accumulated. Among two candidates of carbon sources starch exhibit highest percentage of nitrate reduction with minimum percentage of nitrite accumulation than glucose. The maximum of 100 % removal of nitrate was recorded in the medium supplemented with 0.5% of starch at 60 hours of incubation. Therefore, from the three different percentages of carbon sources tested, 0.5% of starch gave the highest nitrate reduction rate and lower nitrite accumulation regardless of the type of other concentrations.

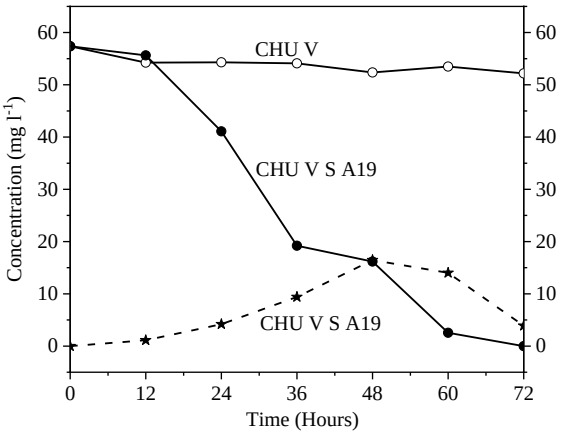


Figure 2. Variation of concentration of nitrate (solid) and nitrite (dot line) with time for the strain *Paenebacillus polymyxa* with 0.5% of starch in water

The result of nitrate removal and the formation of nitrite along with control for the water sample is shown in Figure 2. Similar pattern of nitrate reduction as in the medium was observed in the water treated with strain *Paenibacillus polymyxa*. Nitrate was reduced gradually with the time. Nitrite was accumulated up to 48 hours thereafter gradual reduction was observed. Nitrate was not detected at 72 hours of incubation with 3.89 mgl^{-1} of nitrite accumulation.

Conclusions

Strain A19 was isolated from paddy field and identified as *Paenebacillus polymxa* by biochemical studies and 16s r DNA sequencing analysis. The newly isolated strain *Paenibacillus polymyxa* had high nitrate reduction ability in water, removing nitrate below permissible level in 72 hours of incubation in an aerobic environment, with 3.89 mgl^{-1} nitrite accumulation. It has been shown that the strain is active for efficient removal of nitrate from nitrate rich medium and in water with 0.5 % starch. The results of this study suggest *Paenebacillus polymxa* is a good candidate for aerobic water treatment and 0.5% of starch is the suitable percentage of carbon source for the particular strain. Since it is a non-pathogenic bacteria this could be used for remediation of nitrate contaminated water in commercial level.

Acknowledgement

Financial assistance by National Science Foundation, Sri Lanka under the grant scheme NSF/SCH/2016/03.

References

[1] P.M. Ayyasamy, K. Shanthi, P. Lakshmanaperumalsamy, S.J. Lee, N.C. Choi and D.J. Kim. “Two-stage removal of nitrate from groundwater using biological

and chemical treatments". *Bioscience and bioengineering*, vol. 104, pp. 129-134, 2007.

[2] M. Blaszczyk. "Effect of medium composition on the denitrification of nitrate by Paracoccus denitrificans". *Applied and environmental microbiology*, vol. 59, pp. 3951-3953, 1993.

[3] C.A. Carlson and J.L. Ingraham. "Comparison of denitrification by Pseudomonas stutzeri, Pseudomonas aeruginosa, and Paracoccus denitrificans". *Applied and Environmental Microbiology*, vol. 45, pp. 1247-1253, 1983.

[4] H.J. Ren, Y. Su, C. Wang, Z.M. Hou, Y. Zhou and R. Zhou. "Application of response surface methodology to optimize nitrate removal at low temperature by aerobic denitrificator Pseudomonas strain An-1". *Water and Environment Journal*, vol. 32, pp. 235-241, 2018.

[5] N. Takaya, M.A.B. Catalan-Sakairi, Y. Sakaguchi, I. Kato, Z. Zhou and H. Shoun. "Aerobic denitrifying bacteria that produce low levels of nitrous oxide". *Applied and environmental microbiology*, vol. 69, pp. 3152-3157, 2003.

[6] Z. Wu, S. Huang, Y. Yang, F. Xu, Y. Zhang and R. Jiang. "Isolation of an aerobic denitrifying bacterial strain from a biofilter for removal of nitrogen oxide". *Aerosol and Air Quality Research*, vol. 13, pp. 1126-1132, 2013.

MICROPLASTICS BOUND TRANSPORT OF ANTIBIOTICS IN AQUATIC ENVIRONMENT

T.R. Atugoda*[1, 2] and M. Vithanage[1]

[1]Ecosphere Resilience Research Center, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka
[2]Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka
**Corresponding author (email: thiliatu@yahoo.com)*

Introduction

The global production of plastic exceeds over 300 million tons per year with an annual growth rate of 12 million tons per year. However, only 6-14% of this plastic products are recycled which means the rest is being dumped into landfills or will end up in the natural environment, lakes, rivers and oceans [1]. The large plastic debris known as 'macroplastics' so far has been the common issue disturbing the aesthetic of the marine environment, causing injury and death of marine species resulting from plastic entanglement and ingestion, translocation of non-native species, destroying marine habitats [2] whereas, 'microplastics' have been a significant discovery as a source of marine contaminant. Naturally, macroplastics breakdown to form microplastics through physical and chemical weathering such as UV degradation and mechanical abrasion due to wave and ocean current and microbial degradation. Also microplastics are synthetically produced and incorporated in cosmetics, facial cleansers and personal care products [2]. There are many size ranges to define microplastics but generally it attributes to the plastic particles in the size range of 100 nm to <5 mm diameter.

Microplastics are hydrophorbic and have high surface area to volume ratio which facilitates microplastics to serve as a vector or an organic pollutant carrier through adsorption of various organic contaminant such as persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs) and organochlorine pesticides [3]. However, no studies have been focused on assessing the capacity of microplastics to transport antibiotics, which are emerging contaminants. Antibiotics are being detected in many waste water effluents after wastewater treatment process as many conventional treatment plants have no capacity to remove those[4]. This study is focused on how Polyethylene (PE) microplastics assist binding and transportation of ciprofloxacin hydrochloride, a broad-spectrum synthetic antibiotic used to treat wide variety of bacterial infections at different environmental conditions of varying pH, ionic strength and at the presence of organic carbon etc.

Materials and Methods

Optimum pH determination

A 100 mgL^{-1} CPX stock solution was made by initially dissolving the solid in a minimum amount of methanol and then with de-ionized water. Polyethylene microplastic suspension of 2 gL^{-1} was kept in the shaker for overnight for hydration and dispersion. After spiking 10 ppm of ciprofloxacin, the pH was adjusted in the range of 4 - 9 using 0.1 M HNO_3 and NaOH. At each pH, 10 ml of the sample was drawn out into separate tubes and kept shaking for 12 hours. The final pH was recorded. Samples were filtered using Polytetrafluoroethylene (PTFE) 0.45 µm filter and the absorbance was measured by the Thermo Scientific 10S UV-Vis spectrophotometer at 276 nm wavelength.

FT- IR characterization

The FT–IR analysis was done for PE microplastistics using the Thermo scientific NICOLET iS10 Fourier Transformation Infra-Red Spectrometer in the range of 550-4000 cm^{-1}.

Results and Discussion

FT-IR spectroscopic investigation

The functional groups of PE microplastics were determined by examining the IR spectrum (figure 1). IR peak at ~2916 cm^{-1} for the $-CH_2$ asymmetric stretching, ~2848 cm^{-1} for $-CH_2$ symmetric stretching , ~1463 cm^{-1} for $-CH_3$ symmetric deformation and ~1454 cm^{-1} for wagging were given confirming the chemical characteristics of PE [3].

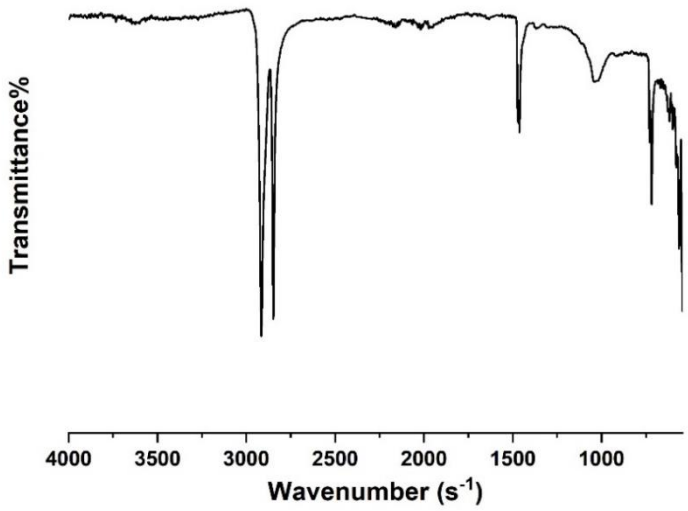


Figure 10. FT-IR microscopy spectrum of polyethylene (PE) microplastics

Effect of pH

The pH range 6 to 7 indicates a maximum adsorption whereas the adsorption capacity decreases towards lower and higher pH values (figure 2). The ionic state of CPX is influenced by different pH depending on its pKa values (pK_{a1}6.1 and pK_{a2} 8.24). At pH below 6, due to the protonation of the -NH group CPX exists in the cationic form and pH above 8 the PCX exists in the anionic form due to the ionization of –COOH group. In between pH 6 and 8 the CPX molecule attains the zwitter ionic state where the net charge is zero[5]. The hydrophorbic interactions between microplastics and CPX become prominent during this pH range exhibiting the highest adsorption of CPX by microplastics.

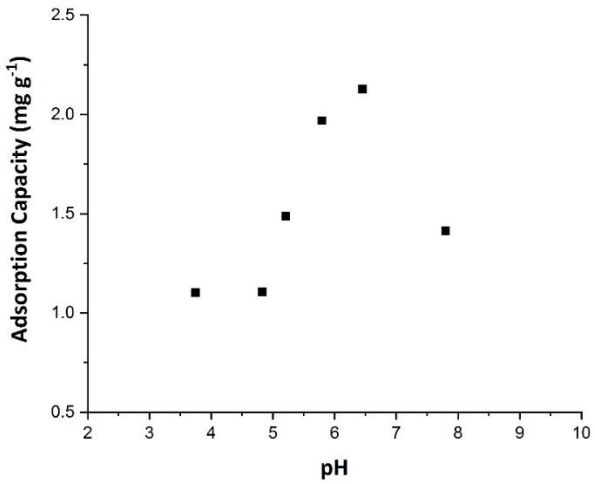


Figure 11. Adsorption capacity of CPX by the sorbent at different pH

Conclusions and Recommendations

Micro plastics are characterized with $–CH_2$ and $-CH_3$ functional groups revealing its non-polar hydrophorbic properties that attribute to PE. These PE microplastics exhibit the highest adsorption capacity of CPX around neutral pH values bearing the potential of mobilizing antibiotic contaminants in the natural aquatic environment. Furthermore, FT-IR analysis for CPX bound microplastics will support further explanation towards the CPX-microplastic interaction. Future studies will be carried out for different ionic strengths (0.1, 0.01 and 0.001 M $NaNO_3$) and in the presence of water soluble organic compounds (Humic acid) to investigate the adsorption of microplastics and the mobility of CPX.

Acknowledgment

Dr. Hasintha Wijesekara and Prof. Nanthi Bolan for providing support for the experiment and Instrument Center of the University of Sri Jayewardenepura for providing facilities for analysis.

References

[1] O. S. Alimi, J. Farner Budarz, L. M. Hernandez, and N. Tufenkji, "Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport," *Environ. Sci. Technol.*, vol. 52, no. 4, pp. 1704–1724, 2018.

[2] M. Cole, P. Lindeque, C. Halsband, and T. S. Galloway, "Microplastics as contaminants in the marine environment: A review," *Mar. Pollut. Bull.*, vol. 62, no. 12, pp. 2588–2597, 2011.

[3] H. Wijesekara *et al.*, "Trace element dynamics of biosolids-derived microbeads," *Chemosphere*, vol. 199, pp. 331–339, 2018.

[4] J. Li, K. Zhang, and H. Zhang, "Adsorption of antibiotics on microplastics," *Environ. Pollut.*, vol. 237, pp. 460–467, 2018.

[5] L. Yang *et al.*, "SERS investigation of ciprofloxacin drug molecules on TiO2nanoparticles," *Phys. Chem. Chem. Phys.*, 2015.

CHANGE IN POLYPHENOLS, CAROTENOIDS AND BIOACTIVITY OF RICE BRAN FERMENTED WITH *Rhizopus oryzae*

G. Janarny* and K.D.P.P. Gunathilake

Department of Food Science and Technology, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila, Sri Lanka.
**Corresponding author (gjanarny3@gmail.com)*

Introduction

Rice (*Oryza sativa*) is the second most common agricultural commodity cultivated around the world especially in developing countries. Nearly 20 million tons of rice is consumed annually in Asian countries. However rice bran is an underutilized by product of rice milling [1].Recently studies have revealed that rice bran is an excellent source of bioactive compounds. It is rich in polyphenols especially phenolic acids like ferullic acids, flavonoids like tricin and some varieties of rice are good source of carotenoids [2].

Epidemiological studies indicate that higher prevalence of chronic diseases like diabetes mellitus and cardiovascular diseases, are mainly due to the oxidative stress occurring within human body. Oxidative stress occurs due to the imbalance between oxidants and antioxidative defense system of human body. Antioxidant compounds like polyphenols play a major role in controlling these chronic diseases and natural antioxidants have gained attention currently due to their additional health benefits like anti-inflammatory and anti-diabetic properties. Thus rice bran which is a potential source of antioxidants play a major role in controlling chronic diseases. Though studies have been done to analyze the antioxidant compounds and their bioactivity in rice bran there are only few studies conducted regarding increasing the bioactive content of rice bran and making it as an effective source of functional ingredient in food industry through processing. This study aims to evaluate the effects of fermentation of rice bran with *Rhizopus oryzae* on its polyphenolic content, carotenoid content and bioactivity. Additionally influence of gastro-intestinal digestion on the bioactive content of rice bran and their bioactivity also was studied.

Materials and methodology

This study was conducted using four varieties of rice bran samples (BG 351, BW 367, BG 406, and H 4) collected from Bathelagoda Rice Research Institute. As described by Anuchita *et al.*, (2012), all rice bran samples were stabilized and the stabilized brans were defatted according to the procedure of Wang *et al.,* (1999).

*Rhizopus oryz*ae required for fermentation was isolated from ripened guava as described by Titik *et al.* (2014). The rice bran was fermented for 96 hours at 30˚C by mixing with the isolated *Rhizopus oryzae* as described by Badiale *et al.* (2007). Extracts of fermented and unfermented rice bran were obtained by simple

solvent extraction using methanol according to the method described by Tan Xiang (2015).

Fermented and unfermented rice bran was subjected to *in vitro* gastro intestinal digestion and dialysis according to the method described by Gunathilake *et al.* (2018). Methanolic extracts and digested fractions of fermented and unfermented rice bran were evaluated for, total phenolic content (TPC) by Folin-Ciocalteau method of Singelton *et al.* (1999), total flavonoid content (TF) by Aluminium chloride method of Zhishen *et al*. (1999), total anthocyanin content (TAC) by pH differential method as described by Giusti and Worlsland (2001), total carotenoid content (TC) according to the method described by Türlerinde *et al.* (1998). Antioxidant capacity of the extracts and digested fractions were analyzed using DPPH radical scavenging assay based on the method of Hatana *et al.* (1988), Total antioxidant capacity based on the method of Prieto *et al.* (1999) and ferric reducing power assay using the procedure of Oyaizu (1986). Anti-inflammatory property was evaluated using the protein denaturation assay described by Gambhire *et al*. (2009) and anti-diabetic properties was evaluated using alpha amylase inhibition assay of Bernefeld, (1955). All assays were carried out with triplicates. Statistical calculations were done by SPSS version 16.0. One way ANOVA was used to determine the statistical differences.

Result and discussion

Table 1. Content of polyphenols and carotenoids in unfermented and fermented rice bran

Rice bran	TPC (mg GAE/g FW)	TFC (mg RE/g FW)	TAC (mg Cy 3-glc/g FW)	TC (mg /g FW)
BW 367				
Unfermented	5.33±1.14 ^{b}	5.20±2.34^{c}	0.10±0.03^{c}	62.49±4.32^{a}
Fermented	8.81±1.24^{a}	14.75±5.76^{a}	1.65±0.14^{a}	71.82±5.23^{a}
BG 352				
Unfermented	4.43±2.12 ^{b}	2.11±0.15^{d}	0.52±0.04^{c}	1.03±0.02^{d}
Fermented	7.89±3.12^{ab}	29.16±3.23^{a}	1.72±0.19 ^{a}	47.61±3.12^{a}
BG 406				
Unfermented	26.88±4.23^{a}	18.44±3.12 ^{b}	9.55±2.32 ^{a}	26.11±6.12^{b}
Fermented	4.13±1.21^{b}	3.09±0.88^{b}	0.01±0.00 ^{a}	3.26±1.12^{b}
H4				
Unfermented	28.96±4.12^{a}	41.11±5.23^{a}	2.97±0.76^{b}	11.48±1.34^{c}
Fermented	6.29±2.12^{b}	7.17±0.22 ^{a}	0.21±0.09^{a}	9.08±2.34^{b}

TPC = Total Phenolic Content, TFC = Total Flavanoid Content, TAC = Total Anthocyanin Content, TC = Total Carotenoid, GAE = Gallic Acid Equivalent, RE = Rutin Equivalent, Cy 3-glc = Cyanidin-3-glucoside, FW = Fresh Weight, Values are expressed as mean±SDValues. Figures with different letters in the same row differ significantly ($p < 0.05$)

Table 2. Antioxidant activities of unfermented and fermented rice bran samples

Rice bran	DPPH (IC_{50}) (mg/L)	FRAP (mg AAE/ g FW)	Total antioxidant capacity (mg AAE/ g FW)
BW 367			
Unfermented	137.39±2.11[a]	3.79±1.11[a]	11.06±2.35[b]
Fermented	65.39±3.35[a]	6.00±2.15[a]	15.35±4.65[a]
BG 352			
Unfermented	35.10±3.3[b]	6.53±1.15[a]	17.08±4.78[a]
Fermented	23.54±6.5[b]	8.73±3.45[a]	24.65±5.12[a]
BG 406			
Unfermented	125.05±11.23[a]	2.71±0.87[a]	24.53±2.56[a]
Fermented	20.16±3.45[b]	0.47±0.03[a]	7.58±1.56[a]
H4			
Unfermented	55.47±6.8[b]	5.63±4.32[a]	14.38±6.7[b]
Fermented	37.98±4.23[a]	0.92±0.01[a]	11.35±2.34[a]

IC= concentration, AAE = Ascorbic Acid Equivalent, FW = Fresh Weight, Values are expressed as mean±SD
Figures with different letters in the same row are significantly different ($p < 0.05$)

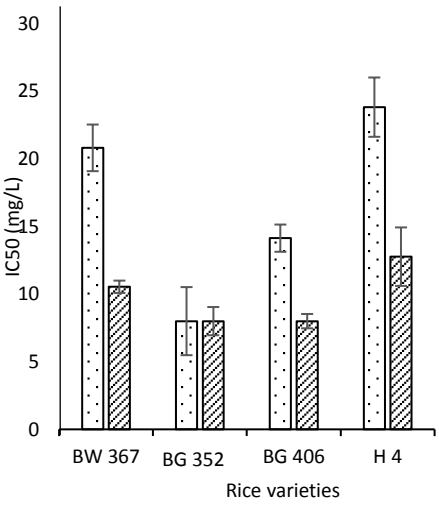


Figure 1. Concentration of fermented and unfermented rice bran extracts at which 50 % of protein denaturation was inhibited. Values are expressed as mean±SD

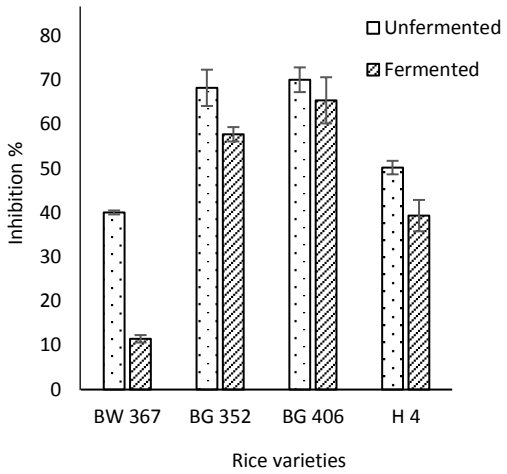


Figure 2. Percentage inhibition of alpha amylase enzyme by fermented and unfermented rice bran samples. Values are expressed as mean±SD

The results obtained for the unfermented and fermented samples were compared to evaluate the changes with fermentation. Accordingly polyphenolic content and carotenoid content of samples has been shown in table 01,

antioxidant activity has been shown in table 02, anti-inflammatory property and anti-diabetic property has been shown in figure 01 and figure 02 respectively.

An increase in the polyphenol content and carotenoid content of BW 367 and BG 352 bran extracts with fermentation can be mainly attributed to the release of bound polyphenols by the extracellular enzymes synthesized during the fermentation by *R.oryzae*.[1] Also synthesis of new phenolic compounds during fermentation causes an increase in the polyphenolic content [1]. Increased polyphenols and carotenoids causes an increase in the antioxidant activity and anti-inflammatory properties of the rice bran extracts [3].

The results from *in vitro* digestion indicated the impact of gastro-intestinal digestion in polyphenols, carotenoids and their bioactivity. Accordingly, in unfermented samples the percentage recovery of total phenols after the gastric digestion ranged from 23.65 % to 84.92%. In fermented samples the percentage of polyphenols recovered after gastric digestion ranged from 63.93% to 82.84 %. The dialyzable phenolic content in unfermented samples ranged from 0.78 % to 1.03% whereas the dialyzable phenolic content of fermented samples ranged from 3.61% to 9.41%. This indicates that fermentation has increased the polyphenol recovery after gastric digestion and dialyzable phenolic content in rice bran samples. Accordingly, in unfermented samples the percentage recovery of carotenoids after the gastric digestion ranged from 9.69% to 58.84%, in fermented samples the percentage of carotenoids recovered after gastric digestion ranged from 35.35% to 78.77 %. This indicates that fermentation has increased the recovery of carotenoids after gastric digestion.

Based on DPPH assay, in the unfermented samples percentage of free radical inhibition at gastric phase ranged from 5.50% to 7.58%. With fermentation, there was a reduction in the radical scavenging ability of all samples in gastric, intestinal and dialyzable fractions compared to the unfermented samples. The reducing power of unfermented samples in the gastric fraction was in the range of 2.81 mg AAE/g Fresh weight (FW) to 3.89 mg AAE/g FW. However there was no significant difference ($P>0.05$) in the reducing power of the dialyzable fractions of all samples. With fermentation reducing power of all samples except BG 406 has increased in the gastric fractions. There was no significant difference ($P>0.05$) in the reducing power of dialyzable fractions of the fermented and unfermented samples of BG 352, BW 367 and BG 406, however a significant ($P<0.05$) reduction was observed in the reducing power of H 4 at its dialyzable fraction. Based on the total antioxidant capacity assay, the results indicate that the total antioxidant capacity in the gastric fractions of unfermented samples were in the range of 7.41 to 8.49 mg AAE/g of FW. However there was no significant differences among the total antioxidant capacity of the dialyzable fractions of the sample. With fermentation total antioxidant capacity of BW 367, BG 406 and H 4 in gastric

fractions have increased. In all dialyzed samples fermentation has increased the total antioxidant capacity.

When considering the changes in anti-inflammatory properties based on the results in both fermented and unfermented samples the protein denaturation inhibition percentage of intestinal and dialyzable fractions were lower compared to the gastric fractions. Inhibition percentage of unfermented samples at the gastric fractions ranged from 1.66% to 6.00% whereas that of fermented samples ranged from 2.50% to 6.92% indicating an increase in the anti-inflammatory properties with fermentation. The anti-inflammatory properties of BG 352, BW 367 and BG 406 in the intestinal phase increased with fermentation compared to the unfermented samples. However in the dialyzable fractions only BW 367 and BG 352 showed an increase in the anti-inflammatory properties with fermentation.

The results indicated that in all fermented and unfermented samples the anti-diabetic properties have reduced in the intestinal fractions and dialyzable fractions compared to the gastric fractions. Among the unfermented samples BW 367 exhibited higher percentage of inhibition in all phases of digestion whereas in fermented samples H 4 exhibited higher percentage of inhibition in all phases of digestion. With fermentation the anti-diabetic properties of BG 352 and BG 406 has increased in all phases of digestion whereas anti-diabetic properties of BW 367 and H 4 has decreased in all phases of digestion.

Conclusion

Fermentation has increased the polyphenolic content, carotenoid content, ferric reducing power and total antioxidant capacity of BW 367 and BG 352. DPPH radical scavenging ability and anti-inflammatory properties of all 4 rice brans have increased with fermentation. However anti-diabetic properties have decreased with fermentation in all 4 rice varieties. Through this study it can be concluded that rice bran is a potential source of bioactive compounds with bioactivity and these can be enhanced using fermentation as a suitable processing technique except for anti-diabetic properties.

Referrences

[1] Schmidt, C. G., Gonçalves, L. M., Prietto, L., Hackbart, H. S., & Furlong, E. B. Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus Rizhopus oryzae. Food Chemistry, [Internet]. 2014, [cited 2018 Feb 08]; 146:371–377. Available from https://doi.org/10.1016/j.foodchem.2013.09.101

[2] Seechamnanturakit, V., Karrila, T. T., Sontimuang, C., & Sukhoom, A. The Natural Pigments in Pigmented Rice Bran and Their Relation to Human Health: A Literature Review. KMUTNB International Journal of Applied

Science and Technology [Internet] 2018 [cited 2018 Feb 09]. Available from https://doi.org/10.14416/j.ijast.2018.01.004

[3] Gunathilake, K.D.P.P., Ranaweera, K.K.D.S.,Antioxidative properties of 34 green leafy vegetables. Journal of Functional Foods [Internet] 2016.26, 176–186.Available at doi:10.1016/j.jff.2016.07.015

FUNCTIONAL PROPERTIES OF FLOUR FROM FIVE DIFFERENT SRI LANKAN CASSAVA (*Mannihot esculenta)* CULTIVARS

R.A.T. Nilusha[1], O.D.A.N. Perera[2], P.I.P. Perera[3], J.M.J.K. Jayasinghe*[1]

[1]Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
[2] Department of Food Science and Technology, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Sri Lanka
[3]Department of Horticulture and Landscape Gardening, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Sri Lanka
**Corresponding author (email: jagathj@sci.sjp.ac.lk)*

Introduction

Cassava (*Mannihot esculenta*) is an important tuber crop and is one of the major sources of carbohydrate that fulfils the dietary requirements of low income families in Sri Lanka. Its physicochemical properties and high availability have made it interesting and challenging ingredient for the food industry. Cassava flour is used extensively in pharmaceutical and food industry because of its unique thickening properties, high purity, low cost and its ability to form clear viscous pastes. In food industry, it is processed into various pre gelatinized instant and convenience foods including gari, pupuru, and fermented cassava flour. It is used in processed baby foods as a filler material and binding agent in confectionary and biscuit industries because of its bland flavor. In Sri Lanka, cassava is cultivated mainly by resource limited farmers only for the starchy roots and Sri Lankans have not explored the potential of cassava in terms of product diversification rather than consumed as boiled or fried chips. Sri Lanka has a surplus production of cassava and it is an unexploited tuber crop while having high demand in both local and export market. Diversification of cassava in to value added products is a promising way to increase the demand and to create a path for resource limited farmers and medium scale entrepreneurs to enter into the local market. Thus, the present study is aimed at investigating the suitability of production of cassava flour from five Sri Lankan cassava cultivars to be used as a raw material in the food industry.

Materials and Methods

Matured tubers of 'Suranimala', 'Swarna', 'Shani', 'MU-51' (var. Peradeniya), and 'Kirikawadi' cultivars of cassava were harvested from the fields of the Horticulture Crop Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka.

Flour Extraction

Cassava tubers were cleaned and peeled. After peeling, they were grated and thoroughly mixed with water in the ratio of 1:1.25, allowed to standing in an open vessel for about 5 h at about 30 ^{o}C. Subsequently, they were dehydrated at 50 ^{o}C for 20 hours. Dehydrated cassava slices were ground and passed through sieves

(0.250mm) to obtain flour. The flour was placed in Poly-Nylon plastic vacuum bags and vacuum packed. Vacuum packed flour packages were stored in food processing laboratory at room temperature until further used [6].

Moisture Content

Moisture contents of flour samples were determined by the oven drying method (AOAC, 2012), by applying the gravimetric principle.

Ash Content

Ash contents of the flour samples were determined by using the dry ashing (AOAC, 2012), by applying the gravimetric principle.

Water Holding Capacity (WHC)

One gram of flour sample was mixed with 10 mL of distilled water and allowed to stand at room temperature (30±2 ^{o}C) for 30 minutes. Then sample was centrifuged for 30 minutes at 3000 rpm. The WHC was expressed as a percentage of grams of absorbed water to weight of the sample [5].

Oil Holding Capacity (OHC)

One gram of flour sample was mixed with 10 mL of refined soy bean oil and allowed to stand at room temperature (30±2 ^{o}C) for 30 minutes. Then sample was centrifuged for 30 minutes at 3000 rpm. The OHC was expressed as a percentage. The OHC was expressed as a percentage of grams of absorbed oil to weight of the sample [5].

Water Solubility Index (WSI) and Swelling Power (SP)

One gram of flour sample was dissolved with 10 mL of distilled water in a graduated centrifuged tube. The suspension was stirred just sufficiently and uniformly avoiding excessive speed since it might cause fragmentation of the starch granules. The slurry in the tube was heated at 85 ^{o}C in a thermostatically regulated temperature water bath for 30 minutes with constant gentle stirring. Then the tube was removed, wiped off the outside of the centrifuged tube and cooled to room temperature. Thereafter, it was centrifuged at 2200 rpm for 15 minutes. The supernatant was decanted into pre weighed moisture can. The solubility was determined by evaporating the supernatant in thermostatically controlled drying oven at 105 ^{o}C and weighing the residue. The sediment paste was weighed and swelling power was calculated as the equation given below [5].

$$\%\ \text{Solubility} = \frac{\text{Weight of soluble}}{\text{weight of sample}} \times 100$$

$$\text{Swelling Power} = \frac{\text{Weight of Sediment}}{\text{Sample wieght-Weight of soluble}}$$

Gelatinization Temperature

One gram of flour sample was weighed accurately in triplicate and transferred to 20 mL screw capped tubes. After adding 10 mL of water into each sample, the

samples were heated slowly in a water bath until they formed a solid gel. At the completion of gel formation the respective temperature was measured and taken as gelatinization temperature [5].

Least Gelation Concentration

Flour dispersions of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20% (W/V) were prepared in 5 mL of distilled water in test tubes and heated for one hour in boiling water (100 ^{o}C) bath. The heated dispersions were cooled rapidly under running tap water and then at 10±2 ^{o}C in a refrigerator for 2 hours. The least gelation concentration was determined from above concentrations when the sample in the inverted tube did not slip [5].

Bulk Density

Twenty grams of flour sample was poured through a short stemmed glass funnel into a 250 mL graduated glass cylinder and the volume occupied by the flour was read and the bulk density calculated in triplicate [5].

Emulsion Activity and Stability

One gram of flour sample was mixed with 5 mL of distilled water and 5 mL of soybean oil in a calibrated centrifuged tube. The emulsion was centrifuged at 3000 rpm for 5 minutes. The ratio of the height of emulsion layer to the height of the mixture was calculated as emulsion activity in percentage. The emulsion stability was determined after heating the emulsion contained in calibrated centrifuged tube at 80 ^{o}C for 30 minutes in a water bath. Cooling for 15 minutes was allowed under running tap water and centrifuged at 3000 rpm for 15 minutes. The percentage of ratio of the height of emulsified layer to the total height of the mixture was expressed as the emulsion stability [5].

Statistical Analysis

All experiments were done in triplicates. The Statistical Package for the Social Sciences (SPSS) version 16.0 package and Microsoft Office Excel 2007 were used in the statistical analyses. In order to compare the means, one way ANOVA was used. In statistical tests, p value < 0.05 was considered significant [5].

Results and Discussion

In the present study, flour types from five different Sri Lankan cassava cultivars were analyzed for physical and functional properties. The results are given in the Table 1.

Table 1: Functional properties of flour from five different Sri Lankan cassava cultivars

	Cassava Cultivar				
Functional Property	**Kirikawadi**	**MU 51**	**Swarna**	**Shani**	**Suranimala**
Moisture (%)	6.56^{a}±0.29	4.45^{b}±0.39	7.78^{c}±0.42	5.35^{d}±0.01	6.49^{a}±0.03

Ash (%)	1.12^{a}±0.02	1.34^{a}±0.11	1.68^{b}±0.04	1.23^{a}±0.08	2.06^{c}±0.01
WHC (%)	308.25^{a}±7.23	275.18^{b}±12.99	211.09^{c}±18.92	$261.29^{b,d,}$±11.16	159.72^{e}±5.61
OHC (%)	110.90^{a}±0.83	$98.82^{a,c}$±3.01	$120.65^{a,b,d}$±8.55	107.53^{a}±12.93	$97.62^{a,c,f}$±5.44
WSI (%)	2.72^{a}±0.19	1.91^{a}±0.25	2.17^{a}±0.08	2.97^{a}±0.01	4.64^{b}±1.44
SP (%)	6.79^{a}±0.1	16.43^{b}±0.99	8.09^{c}±0.42	$10.80^{d,f}$±0.33	$10.40^{e,f}$±0.39
LGC (%)	10^{a}%	8^{a}%	8^{a}%	4^{b}%	6^{c}%
GT (^{o}C)	68.33^{a}±0.58	68.33^{a}±0.58	68.00^{a}±1.00	68.33^{a}±0.57	68.61^{a}±0.58
BD(g/mL)	2.03^{a}±1.15	2.17^{a}±2.89	2.08^{a}±0.57	2.13^{a}± 2.12	2.28^{a}±0.71
EA (%)	52.54^{a}±0.54	$62.37^{a,b}$±5.04	53.53^{a}±0.01	$44.62^{a,c}$±8.56	$45.87^{a,c,d}$±2.86
ES (%)	45.96^{a}±1.14	48.953^{a}±0.61	46.96^{a}±0.81	46.94^{a}±0.54	44.66^{a}±2.86

WHC - Water Holding Capacity, OHC -Oil Holding Capacity, WSI -Water Solubility Index, SP -Swelling Power, LGC -Least Gelation Concentration, GT -Gelatinization Temperature, BD **-** Bulk Density, EA - Emulsion Activity, ES – Emulsion Stability
Values are expressed as "Mean±SD" of three independent determinations.
Different superscripts in a raw represent significantly different samples.

There was no significant difference between the moisture contents of flour from Kirikawadi and Suranimala cultivars (*P>0.05)* while all other cultivars had significant differences in moisture contents of flour. They were ranged between 4.45±0.39% to 7.78±0.42%. Flour from MU 51 cultivar had the lowest moisture content while flour from Swarna cultivar had the highest moisture content. Maximum ash content of 2.06±0.01% was reported in Suranimala cultivar while Kirikawadi cultivar had the minimum ash content. Ash contents of flour from five cultivars had significant differences between them except between Kirikawadi and MU 51 cultivars (*P>0.05)*, Kirikawadi and Shani cultivars (*P>0.05)* and MU 51 and Shani cultivars (*P>0.05)*. Highest WHC of 308.25±7.23% and OHC of 120.65±8.55 were found in flour from Kirikawadi cultivar and Swarna cultivar respectively. The lowest water holding and oil holding capacities were found in flour from Suranimala cultivar. In case of WHC of flour, there was no significant difference between MU 51 and Shani cultivars (*P>0.05)* while all other had significant differences in their WHCs. Swarna cultivar had significant differences of OHC with MU 51 (*P<0.05)* and Suranimala (*P<0.05)* cultivars while all other cultivars did not show significant differences in OHCs. The differences in WHC can be attributed to their different protein fractions. Flour with high WHC can be used in formulation of foods such as sausages, processed cheese and bakery products. OHC is mainly due to the physical entrapment of oil by capillary attraction [3]. Moreover, the hydrophobicity of proteins plays a major role in fat absorption [4]. Among the flour, variations in OHCs might be partially different due to different proportions of nonpolar side chains of the amino acids on the surfaces of their protein molecules. High OHC of flour is potentially useful in flavor retention, improvement of palatability and extension of shelf life in meat products. The SP and WSI of cassava flour from different cultivars were ranged from 6.79±0.1% to 16.43±0.99% and 1.91±0.25% to 4.64±1.44% respectively. The WSI of Suranimala cultivar had significant differences between Kirikawadi (*P<0.05),* MU 51 (*P<0.05)*, Swarna (*P<0.05)*, and Shani (*P<0.05)* cultivars. There was no significant difference

between the SP of Shani and Suranimala cultivars (*P>0.05)* whereas all other cultivars had significant differences in SP values. SP and WSI provide evidence of the magnitude of the interaction between starch chains within both the amorphous and crystalline domains [1]. Higher WSI and SP at higher temperature have important role in determining textural, pasting and thickening properties of starch based preparations. Therefore, cassava flour from these cultivars show a high potential to be used for the applications similar to the above in the food industry. Gelation is an aggregation of denatured molecules and the least gelation concentration is defined as the lowest protein concentration at which gel remained in the inverted tube. Flour from Kirikawadi cultivar formed the gel at a higher concentration (10%) and flour from Shani cultivar formed gel rapidly at lowest concentration (4%). Shani and Suranimala cultivars had significant difference (*P<0.05)* in LGC between them and with other cultivars. The gelation capacity is attributable to the globulin fraction and have been suggested that this property would make the proteins useful in food systems, e.g., puddings and sauces which require thickening and gelling properties. GT of flour was ranged from 68±1.00 ^{o}C to 68.61±0.58 ^{o}C. High GT may be due to the high amylopectin content where the branches prevent the degree of association for gel formation [2]. The BDs of five flour types were ranged between 2.28±0.71 g/mL and 2.03±1.15 g/mL. High BDs indicates that it would serve as good thickeners in food preparations. There were no significant differences in GT or BD of five flour types. The EA and ES of five types of cassava flour were ranged between from 62.37±5.04% to 44.62±8.56% and 48.53±0.61% to 44.66±2.86% respectively. The MU 51 cultivar had significant difference in EA with Shani cultivar (*P<0.05)* and Suranimala cultivar (*P<0.05)* while other had no significant differences between them. These high EA and ES of cassava flour can be used as primary functional properties in foods such as salad dressings, frozen desserts and mayonnaise.

Conclusions and Recommendations

According to the present study, it can be concluded that there is an effect of cultivar on the functional properties of the cassava flour. Moreover, the flour from Kirikawadi, MU 51, Swarna, Shani and Suranimala cultivars can be used as raw materials in the food industry. The present findings revealed that the potential of extraction and utilization of flour from Sri Lankan cassava cultivars for its commercial applications in various food industries.

References

[1] I.P. Claver, H. Zhang, Q. Li, K. Zhu and H. Zhou, “Impact of the Soak and the Malt on the Physicochemical Properties of the Sorghum Starches”. *International Journal of Molecular Sciences,* Vol.11, pp. 3002-3015, 2010

[2] T. Gaffa, Y. Yoshimoto, I. Hanashiro, O. Honda, S. Kawasaki and Y. Takeda, “Physicochemical properties and molecular structure of starches from millet

(Pennisetum typhoides) and sorghum (Sorghum bicolor L. Moench) cultivars in Nigeria". *Cereal Chemistry,* Vol. 81, pp.255-260, 2004

[3] J.E. Kinsella, "Functional properties of protein in food- A survey". *Critical Review Food Science Nutrition*, Vol. 5, pp. 219, 1976

[4] L.P. Voutsinas and S. Nakai, "A simple turbidimetric method for determining the fat binding capacity of proteins". *Journal of Agricultural and Food Chemistry*, Vol. 31, pp. 58-61, 1983

[5] C. Suresh and Samsher, "Assessment of functional properties of different flours". *African Journal of Agricultural and Food Chemistry,* Vol. 31, pp. 58-61,1983

[6] S. Samad, H. Rasulu, Hasbullah and S. Hasan, "Chemical properties of high quality cassava flour (HQCF) from several varities of cassava". *Pakistan Journal of Nutrition*, Vol.17, pp.615-621, 2018

GLYCEMIC INDEX AND GLYCEMIC LOAD OF SELECTED *Ipomea batatas* (SWEET POTATOES) CULTIVARS AVAILABLE IN SRI LANKA

G.R.N.N. Waidyarathna* and S. Ekanayake

Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
**Corresponding author (email:nipuni_nayanathara@yahoo.com)*

Introduction

Glycemic index (GI) of a food is defined as determined by quantifying the blood glucose response to an ingested quantity of carbohydrate in a food as compared to the response using a standard reference food containing the same amount of carbohydrate [1] and the glycemic load (GL) is the product of the GI and the amount of available carbohydrate in a specific portion of food [2] as the volume of food used for GI determination may sometimes not reflect an edible portion. Sweet potato is a low input tuber crop, which has a short production cycle and adaptability to less favorable conditions. Also sweet potatoes are rich in minerals, vitamins, dietary fiber and bioactive compounds such as phenolic compounds and anthocyanin. Furthermore, varieties with yellow colored flesh are a good source of carotenoids, which has reported contributes as improved the vitamin A status of children. Although there are more than 20 varieties of sweet potatoes are available in Sri Lanka, glycemic index and the glycemic load of most of these varieties are not yet reported. The objective of this study is to make available data on glycemic index and glycemic load of some selected Ipomea batata (sweet potatoes) cultivars available in Sri Lanka.

Materials and Methods

Sample selection

Commonly available and selected improved sweet potato varieties were selected for the study and they are namely Wariyapola Red, Ranabima, CARI 426 and CARI 273. Samples were obtained from Plant Genetic resource center, Gannoruwa.

Sample preparation

Sweet potato flour samples were prepared for the determination of digestible carbohydrate content. The flour preparation was done by boiling (as home cooking), oven drying (40-45oC), milling (by analytical mill) and sieving (100 mesh).

Determination of digestible carbohydrate content

The prepared sweet potato flour was used for the determination of digestible carbohydrate of each variety analysis and AOAC standard methods were used (Megazyme total starch assay kit) for the determination of digestible carbohydrates of each tested variety.

Portion size determination

Portion sizes of sweet potatoes for the GI study were calculated to provide 25g of digestible carbohydrates from each portion. The moisture content of sweet potato flour and freshly boiled sweet potatoes, which were determined using AOAC standard methods, were used for the calculations.

Determination of glycemic index values

WHO standard procedure, with a sample size of 10 individuals was used for the study [3], [4]. Glucose was used as the reference food. Apparently healthy men and women who are not undergoing any medical treatment with a normal BMI (18.5 -23) and age between 18-30 years were selected for the study. After obtaining a blood sample following an 8-10 hour fast, 25g glucose /sweet potatoes (with 25g of digestible carbohydrates) were given to ingest thrice on three different days to each subject after an overnight fast of ~ 8-10 hours during a 15 minutes period. Capillary blood was drawn at 0, 30, 45, 60, 90 and 120 minute intervals and the blood glucose concentrations were measured using the glucose oxidase kit method. Same procedure was followed on different days with each sweet potato containing 25g of digestible carbohydrate.

GI values were determined for each cultivar using the incremental area under the curve as the incremental area under the post prandial blood glucose response curve (IAUC) ignoring the area beneath the fasting concentration against the IAUC of standard. GI was obtained by applying the values in the equation given below.

$$GI = (IAUC\ sample / IAUC\ standard) \times 100$$

Each GI value of 10 individuals was averaged to calculate the GI of the each cultivar.

Glycemic load determination:

Digestible carbohydrate content in the given portion and average GI values of each variety were used for the GL determination.

Also edible portion sizes of each variety were determined using a questioner provided to study subjects and the digestible carbohydrate was calculated for the edible portion sizes. GL was calculated using the equation stated below.

GL was calculated using below equation

GL = GI * Digestible carbohydrate in edible portion/ 100

Ethical Clearance:
Ethical Clearance was obtained from the ERC of University of Sri Jayewardenepura (No 48/15).

Results and Discussion
Digestible carbohydrate and the moisture contents of freshly boiled sweet potatoes varied between 6%-8% and 77%- 81% respectively. GI values and GL values of each variety are presented in Table 1.

Table1. Glycemic index and glycemic load values of the selected *Ipomea batata* (sweet potatoes) cultivars. (± Standard error of mean)

Variety	Glycemic index	Glycemic load	
		for portion given for GI	for edible portion
Wariyapola Red	101±7	25	13
Ranabima	120±6	30	15
CARI 426	97±11	24	12
CARI 273	104±7	26	13

All studied sweet potato cultivars were elicited high GI values. High moisture content in boiled sweet potatoes may increase starch gelatinization and high amylopectin content [5] may be reasons contributory for having high GI values. GL of the portion taken for GI with 25g of digestible carbohydrates have elicited high GL but as all the subjects were informed as that the given portion sizes were too large to consume., When considering the an actual edible portion for as reported by the volunteers (1/2 of the portion given for GI study) were considered and (obtain from a questioner given to the subjects participated for GI study) accordingly all the tested sweet potato varieties have elicited medium GL. High moisture content of all varieties reduce the volume of edible portion and thus carbohydrate intake, the major nutrient.

Conclusions and Recommendations
All studied sweet potato cultivars were thus categorized as high GI foods. When considering the portion with 25g of digestible carbohydrates, all the studied

varieties have shown high GLs but considering the actual edible portion sizes, GL of all the varieties were medium. As sweet potatoes are elicit high GI food; this flour can be utilized to prepare foods to fulfill the energy requirement of malnourished individuals, athletes and the growing children who require energy dense food products.

References

[1] D.J. Jenkins, T.M.S. Wolever, R.H. Taylor, H. Barker, H. Fielden, J.M. Baldwin, J.M., A.C. Bowling, H.C. Newman, A. L. Jenkins and D.V. Goff. "Glycemic index of foods: a physiological basis for carbohydrate exchange", American Journal of Clinical Nutrition, vol. 34 (3), pp. 362–366.1981.

[2] J. Salmeron, J.E. Manson, M.J. Stampfer, G.A. Colditz, A.L. Wing and W.C. Willett. "Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women", Journal of the American Medical Association, vol. 277 (6), pp. 472–47.1997.

[3] FAO/WHO. "Carbohydrates in human nutrition. Report of a Joint FAO/WHO Expert Consultation". FAO Food Nutr Pap; 66:1–140. 1998.

[4] F. Brouns, I.Bjorck, K.N. Frayn, A.L. Glibbs, V. Lang, G. Slama and T.M.S. Wolever. "Glyceamic index methodology. Nutrition Research Reviews", vol. 18, pp. 145-171. 2005.

[5] S.A. Senanayake, K.K.D.S. Ranaweera, A. Gunaratne and A. Bamunuarachchi. "Comparative analysis of nutritional quality of five different cultivars of sweet potatoes (Ipomea batatas (L) Lam) in Sri Lanka", Food Science and Nutrition, vol.1 (4),pp. 284-291. 2013.

Acknowledgement

- Financial assistances by University of Sri Jayewardenepura - ASP/01/RE/MED/2015/48.
- Research officers & the staff members of Plant Genetic Resource Center, Gannoruwa.
- Volunteers who participated in the GI study.

GLYCEMIC INDICES AND GLYCEMIC LOADS OF SOME SELECTED TRADITIONAL (*Oryza sativa L.)* RICE VARIETIES

K.A.D.L Bhagya* and S. Ekanayake

University of Sri Jayewardenepura, Gangodavila, Nugegoda
**Corresponding author (email: lakminibhagya@gmail.com)*

Introduction

Rice is the dietary staple and the major source of energy in a Sri Lankan diet. Rice irrespective of variety comprises of 75-80% starch [4]. Hence due to the prevalence of non communicable diseases in the country the consumption of high proportion of rice indicates the need for investigating the bioavailability and metabolic responses of major sources of starch. As there is no sufficient data on metabolic response to traditional rice varieties this study aims to investigate the Glycemic indices (GI) and Glycemic loads (GL) of some traditional rice varieties. The glycemic index is defined as the incremental area under the blood glucose response curve of a 50 g digestible carbohydrate portion of a test food expressed as a percent of the response to the same amount of carbohydrate from a standard food taken by the same subject [1] while GL is the product of a food's GI and its total available carbohydrate content [3]. While GI is a reflection of quality, GL reflects the actual glycaemic load of an edible portion of a starchy food.

Materials and Methods

Sample selection

Traditional rice samples were selected, authenticated and obtained from Bathalegoda Rice Research Institute. Selected rice varieties were milled but not polished and namely Kuruluthuda, Pokkali, Suwadel, and Pachchaperumal. Except Suwadel all the selected varieties were red rice.

Sample preparation

Rice samples were prepared for digestible carbohydrate determination following cooking, drying (55ºC), milling (analytical mill) and sieving (100 mesh) to obtain whole rice flour.

Digestible starch content was determined using the megazyme starch assay kit method. Starch content was determined as per the equation mentioned below.

$$Starch\% = \frac{\Delta A * F * FV * 0.9}{W}$$

Determination of Glycemic index

Portion sizes of each rice for the GI study were calculated so that rice provided 50 g of digestible carbohydrate per portion. Apparently healthy men and women not undergoing any medical treatment with normal BMI (18.5-23) and aged between 18-30 years (10 subjects) who volunteered were selected for the study. Subjects were given the standard (50g glucose) after an overnight fast (8-10 hours) and after obtaining a fasting blood sample, to consume during 15 min with 250 mL of water. Further capillary blood samples were collected by finger prick at 30, 45, 60, 90 and 120 minute intervals following ingestion of glucose. Blood glucose concentrations were measured using the glucose oxidase kit method. Selected cooked *Oryza sativa* varieties containing 50 g of digestible starch was given to the above volunteers on subsequent days with a gap between days. Capillary blood was drawn and testing was carried out as in above mentioned procedure.GI values were determined for each variety using the incremental area under the curve (IAUC) against the IAUC of standard. GI was obtained by applying the values in the equation given below. The average GI of 10 individuals for each rice variety was taken as the GI of each rice variety [2].

$$GI = (IAUC\ sample / IAUC\ standard) \times 100$$

Determination of Glycemic load

Glycemic load was calculated after obtaining the glycemic index value by applying in the equation given below.

$$glycemic\ load = [GI \times carbohydrate\ (g)\ in\ edible\ portion]/100$$

Results and Discussion

Moisture, digestible carbohydrate content, portion sizes, GI and GL of studied rice are stated in Table 1.

Table 1. Moisture, digestible carbohydrate content, portion sizes, GI and GL of some selected traditional rice varieties

Variety	Moisture of cooked rice (N=5)	Digestible carbohydrate (100 g freshweight) (N=4)	Portion size (g)	Glycemic index (N=10)	Glycemic load
Kuruluthuda	61.8±0.5^{a}	26.6± 0.9^{a}	171	85± 5	42
Pokkali	60.3± 2.2^{a}	30.9± 1.9^{bd}	150	80± 8	40
Suwadel	55.9± 1.0^{b}	33.9± 1.9^{b}	147	84± 9	42
Pachchaperumal	61.7± 2.5^{a}	30.3± 1.7^{cd}	159	80± 6	40

Data represented as mean ± SD. Mean values in the column superscripted by different letters were significantly different at $p < 0.05$.

Moisture contents of cooked rice ranged between 56% to 62%. Suwadel was found to have significantly low moisture content ($P< 0.05$). Digestible carbohydrate content of rice on fresh weight basis varied from 27% to 34%. Suwadel the white rice variety had the highest digestible carbohydrate content. GI ranged between 80-85 indicating the selected traditional rice varieties belonged to high GI category. Portion sizes given for consumption varied from 147 g to 171 g. The edible protions of all varieties given for consumption were considered as adequate by the volunteers. Thus the glycemic loads provided by all rice were high and ranged between 39-42.

Conclusions and Recommendations

Irrespective of rice being red or white glycemic indices and glycemic loads of the studied traditional rice were categorized as high GI and high GL. Consumption of white or red rice with accompaniments which will reduce the portion size of rice will lead to low glycemic load intake.

References

[11]FAO/WHO. “Carbohydrates in human nutrition. Report of a Joint FAO/WHO Expert Consultation”. *FAO Food Nutr Pap*; 66:1–140. 1998.

[12]F. Brouns, I. Bjorck, K.N. Frayn, A.L. Glibbs, V. Lang, G. Slama and T.M.S. Wolever. “Glyceamic index methodology. Nutrition Research Reviews”, vol. 18, pp. 145-171. 2005

[13]J. Wigdon “Glycemic index and glycemic load. Internet: www.lpi.oregonstate.edu/mic/food-beverages/glycemic-index-glycemic-load 2018 [Aug. 28, 2018]

[14]P.M.H.D Pathiraje, W.M.T Madhujith, A. Chandrasekara, and S.P. Nissanka “The effect of rice variety and parboiling on in vivo glycemic response”. Tropical Agricultural Research, vol 22, pp 26-33, 2010

Acknowledgement

Financial assistances by University of Sri Jayewardenepura-ASP/01/RE/MED/2016/51.

IN VITRO ANALYSIS OF GASTROINTESTINAL TOLERANCE OF PROBIOTIC (*lactobacillus* SPECIES) IN ARROWROOT (*Maranta arundinacea*) INCORPORATED SYNBIOTIC ICE-CREAM

P.M. Kumarage* and S. Jayathilake

Department of Food Science and Technology, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), Sri Lanka
**Corresponding author (email:* prasangamadushani@gmail.com*)*

Introduction

Recently, development of probiotic microorganisms incorporated functional foods have been increased due to the beneficial effects of probiotics. Among these functional food products, synbiotic products have high demand due to the presence of both probiotics and prebiotics. Ice-cream is one of the good sources for probiotics due to its neutral pH, because low pH will affect the viability of probiotics [1]. Arrowroot (*Maranta arundinacea*) is a rich source of fructooligosacchrides which has prebiotic effect. Therefore, arrowroot incorporated synbiotic ice-cream was developed as a new synbiotic ice-cream. However, benefits of probiotics can only be met if there are 10^6- 10^7 cfu/g in the product at the time of consumption. As well as they need to be survived inside the gastrointestinal tract while tolerating acid, bile and enzymes after the consumption to exert their beneficial effects[2]. Therefore, several studies have been investigated to evaluate viability of probiotics in products under the gastrointestinal tract using *in vivo* and *in vitro* methods. *in vitro* simulated gastrointestinal tolerance assay was frequently suggested for the evaluation of the probiotic potential in digestive environment. Therefore, several studies have been investigated to evaluate viability of probiotics in products using *in vitro* simulated gastrointestinal tolerance assay. Products are digested by creating a digestive environment using enzymes, alkaline solution, acid during the *in vitro* simulated gastrointestinal assay. However, there are limited studies on gastrointestinal tolerance of probiotics in arrowroot incorporated ice-cream. Therefore, Main objective of this research study was to evaluate the gastrointestinal tolerance of probiotic (*Lactobacillus* sp.) in arrowroot incorporated synbiotic ice-cream. Thereby the impact of arrowroot extract on the viability of *Lactobacillus* sp. was evaluated under the *in vitro* simulated gastrointestinal conditions throughout the 55 days of storage period at -18 ^oC. Also, the effect of ice-cream as a food matrix was evaluated compared to fresh probiotic culture.

Material and Methods

Extraction of prebiotic from raw arrowroots

Extraction of prebiotics from arrowroots was carried out according to the method described earlier [3] with modifications. Sorted and washed raw

arrowroots were cut into small pieces and were blended. Then, they were mixed with distilled water in 1:5 ratio (Arrowroot paste: distilled water). Mixture was allowed to settle for 30 minutes and filtered out. Filtrate was allowed to settle another 1 hour and precipitate was separated. Precipitated arrowroot extract was dried at 50 ^{o}C for 24-36 h using an electric oven. Dried precipitate was blended into fine powder, vacuum packed and stored under refrigeration conditions (4 ^{o}C).

Incubation of probiotics

13% (w/v) of skim milk solution was prepared using sterilized distilled water. Then ABY- 3 (La-5, Bb-12 and *Streptococcus thermophilus*) freeze dried probiotic yoghurt culture (0.1% (w/V)) was incorporated into 13% skim milk solution. After that it was incubated for 16 hours at 37 ^{o}C.

Preparation of coconut milk substituted control, arrowroot and reference ice-cream

Arrowroot synbiotic ice cream (with 7% (w/v) of arrowroot extract), probiotic ice cream without prebiotics (control), ice cream with 7%(w/v) inulin (reference) were prepared according to previous research study (unpublished).

Fresh milk and coconut milk were mixed in 60:40 ratio respectively (initial mixture). In order 23% (w/v) of white sugar, 2 drops of vanilla essence, 16%(w/v) of whipping cream, 1%(w/v) of gelatin and 0.5%(w/v) of cremodon 30 (ice-cream stabilizer) were added into initial mixture and dissolved well by beating for 2 minutes after addition of all ingredients. Final mixture was kept in the freezer at -18 ^{o}C for 30 minutes. After 30 minutes, mixture was taken out and beat for 10 minutes. Then this step was repeated for five times with intermittent freezing. After that, final mixture was separated into three portions to prepare control, arrowroot and reference ice-cream.

Control ice-cream was prepared by adding 10% of previously incubated probiotic culture at the last beating during intermittent freezing. After addition of incubated probiotic culture into another two portions of final ice-cream mixture, arrowroot synbiotic ice-cream and reference ice-cream were prepared by adding 7 % (w/v) arrowroot extract and 7 % (w/v) inulin into that two portions of final ice-cream mixture separately at the last beating. Finally, three types of ice-cream mixtures were poured in to 80 mL ice-cream cups separately and stored at -18 ^{o}C in freezer for 12 hours and store at same temperature for further analysis. Ice cream samples were used to determine the gastrointestinal tolerance of *Lactobacillus* sp. after 1,13,25,41 and 55 days of frozen storage. All tests were done in duplicates.

In vitro simulated gastrointestinal tolerance assay for Lactobacillus sp. in ice-cream samples

The tolerance of *Lactobacillus sp.* to *in vitro* simulated gastric and enteric conditions was performed according to the method described by [4]with slight modifications.

Initially, ice-cream samples (25 g) were mixed in 225 mL of 0.5 g/100 mL NaCl solution in separate sterilized beakers. Then 10 ml aliquots from each sample were taken into three separate sterilized tubes. For gastric phase simulation, pH of aliquots (10 mL) was adjusted to 2.1- 2.6 with HCl (0.5 mol/L) and pepsin was added into samples to obtain a concentration of 3 g/L. Then tubes were incubated at 37 ^{o}C for 2 h with agitation approximately 100 rpm (Daihan labtech, Korea).
After 2h, in order to simulate enteric phase 1, pH of samples was increased up to 4.9-5.4 using an alkaline solution (150 mL of 1 mol/L NaOH solution, 14 g of $PO_4H_2Na.2H_2O$ and distilled water up to 1 L). Then bile salt and pancreatin were added to obtain a concentration of 10 g/L and of 1 g/L, respectively. After that, samples were incubated at 37 ^{o}C for 2 h under agitation. After 4 h, pH of samples were increased to 7.5-7.7 using same alkaline solution, bile and pancreatin were added to maintain the concentrations of 10 g/L and of 1 g/L respectively, and the samples were incubated again at 37 ^{o}C for 2 h under agitation, leading to enteric phase 2 and achieving 6 h of assay. Finally, enumeration of *Lactobacillus sp*. was performed in aliquots collected from samples after 0,2,4 and 6 h by spread plating in MRS agar followed by the incubation at 37 ^{o}C for 48 h.

In vitro gastrointestinal tolerance assay for fresh probiotic culture
In vitro gastrointestinal tolerance assay of fresh probiotic culture was carried out according to the method describe in [5]. An ABY-3 freeze dried culture,0.1 g was grown in 100 mL of sterilized MRS broth at 37 ^{o}C for 18 h. After that cells were harvested by centrifugation at 4,000 rpm, at 4 ^{o}C, for 10 min. After that, supernatant was removed. Then harvested cells were resuspended in 100 mL of sterile 0.5% (w/v) NaCl solution. After that, 10 mL aliquot was collected into sterilized tubes from that suspension for the *in vitro* gastrointestinal tolerance assay. finally, enumeration of fresh *lactobacillus sp*. was done as pervious. Preparation of fresh culture was performed in duplicates.

Enumeration of Lactobacillus sp in MRS agar
After 2 h, 4 h and 6 h of *in vitro* gastrointestinal tolerance assay 1 ml of each ice-cream aliquots (control, sample and reference) and fresh probiotic cell suspension were collected, in to four sterilized distilled water tubes separately and serial dilutions were prepared up to 10^{-6} respectively. Then population of *Lactobacillus* sp*.* were enumerated by spread plating technique using 0.1 mL of each dilution in MRS agar followed by incubation at 37 ^{o}C for 48 h. Each steps were done under aseptic conditions. The results were expressed as log cfu/g. Enumeration of *Lactobacillus* sp*.* in spread plates were done in duplicates.

Result and Discussion

All data were analyzed using factorial one-way ANOVA in SPSS 16. Results showed that cell viability of *Lactobacillus* sp. ranged from 7.7 to 6.5 log cfu/g of three types of ice-cream during the 55 days of frozen storage before digestion (Figure 1). The lowest probiotic cell viability was recorded for control ice-cream than arrowroot and reference ice-cream, but there was no significant difference ($p<0.05$) in probiotic cell viability of all three types of ice-cream. Probiotic cell viability decreased to around 5.5 to 4.5 log cfu/g in three types of ice-cream at the end of 2 h of digestion throughout 55 days of storage. It can be concluded probiotics high sensibility toward simulated gastric juice containing HCl and pepsin. The lowest probiotic cell viability was recorded for control ice-cream when compared to arrowroot and reference ice-cream. Probiotic cell viability in control ice-cream was significantly lower ($p<0.05$) than probiotic cell viability in arrowroot and reference ice-cream. Average probiotic cell viability in arrowroot, control and reference ice-cream were 5.73, 5.30 and 5.66 log cfu/g respectively at the end of the *in vitro* assay throughout the 55 days of storage period (Figure 2) and probiotic cell viability was significantly lower ($p<0.05$) in control ice-cream than probiotic cell viability in arrowroot and reference ice-cream. As well as the cell viability in fresh culture was significantly lower ($p<0.05$) than cell viability in three types of ice-cream at the end of the *in vitro* assay during the 1st day of frozen storage (Figure 3), suggesting that ice cream food matrix positively improved the probiotics cell viability when compared to fresh probiotic culture.

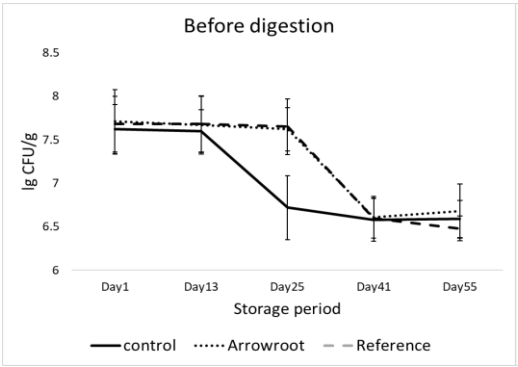


Figure1. Probiotic cell viability before digestion throughout the storage period

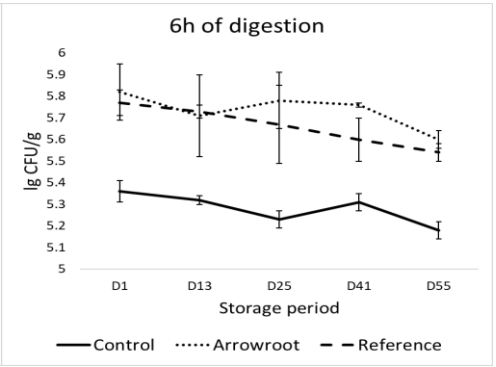


Figure2. Probiotic cell viability after 6h digestion throughout the storage period

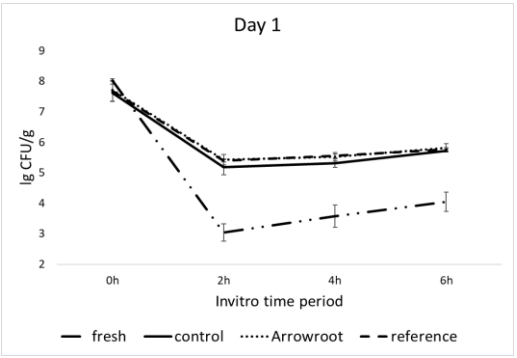


Figure 3: Probiotic cell viability under gastrointestinal conditions on 1st day of frozen storage

Conclusion and Recommendations

This research study concluded that arrowroot synbiotic ice-cream positively improved the survivability of *Lactobacillus* sp. because the presence of arrowroot extract positively effect on the survivability of *Lactobacillus* sp when compared to control ice-cream by the end of the *in vitro* assay. And also, ice cream food matrix can be considered as a good vehicle for *Lactobacillus* sp. when compared to fresh culture and could play a vital role in probiotic protection against gastrointestinal conditions. As further studies this research study can be extended to investigate the survivability of pure strain of *Lactobacillus* sp. in arrowroot synbiotic ice cream under the *in vitro* and *in vivo* gastrointestinal conditions.

References

[1] A. G. Cruz, A. E. C. Antunes, A. Lúcia, O. P. Sousa, J. A. F. Faria, and S. M. I. Saad, "Ice-cream as a probiotic food carrier," *Food Res. Int.*, vol. 42, no. 9, pp. 1233–1239, 2009.

[2] A. R. Madureira, C. I. Pereira, K. Truszkowska, and A. M. Gomes, "Survival of probiotic bacteria in a whey cheese vector submitted to environmental conditions prevailing in the gastrointestinal tract," *Int. Dairy J.*, vol. 15, pp. 921–927, 2005.

[3] N. Abesinghe, J. Vidanarachchi, and S. Silva, "The Effect of Arrowroot (*Maranta arundinacea*) Extract on the Survival of Probiotic Bacteria in Set Yoghurt," *Int. J. Sci. Res. Publ.*, vol. 2, no. 1, pp. 2–5, 2012.

[4] P. Diniz, M. De Fátima, K. Maria, R. Targino, and P. Correia, "LWT - Food Science and Technology Potentially probiotic ice cream from goat ' s milk : Characterization and cell viability during processing , storage and simulated gastrointestinal conditions," *LWT - Food Sci. Technol.*, pp. 1–6, 2014.

[5] R. Bedani, E. Antonio, S. Marta, and I. Saad, "Impact of inulin and okara on Lactobacillus acidophilus La-5 and Bi fi dobacterium animalis Bb-12 viability in a fermented soy product and probiotic survival under in vitro simulated gastrointestinal conditions," *Food Microbiol.*, vol. 34, no. 2, pp. 382–389, 2013.

COMPARISON OF ANTIBACTERIAL ACTIVITY OF SRI LANKAN BEE HONEY WITH NEW ZEALAND MANUKA HONEY IN RELATION TO THE BOTANICAL ORIGIN AND PHYSICOCHEMICAL PROPERTIES

H.G.Y.R. Silva*[1], R.A.C.H. Seneviratne[1], M. Gunawardana[2], C.V.L. Jayasinghe[1]

[1]*Department of Food Science & Technology, Faculty of Livestock Fisheries & Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawilla, 60170, Sri Lanka*
[2]*Sri Lanka Institute of Nanotechnology, Nanotechnology & Science Park, Mahenwatta, Pitipana, Homagama, 10206, Sri Lanka*
**Corresponding author (email: yashodyaranganie@gmail.com)*

Introduction

High acidity, low pH, low moisture, high total soluble solids (TSS), other chemical compounds and botanical origin of honey are highly associated with the antimicrobial properties of honey [1]. Manuka honey originates from Manuka tree which is native to New Zealand exhibits antibacterial activity towards antibiotic resistant pathogens. Therefore it is used to treat patients with chronic wounds. Its non-peroxide antimicrobial activity is mainly due to the chemical compound called methylglyoxal (MGO). Manuka honey is quite expensive and has higher demand than Sri Lankan bee honey. Approximately 10,000 tons of Manuka honey are being sold internationally every year. Although Sri Lankan bee honey has being exhibited good medicinal properties, it has a lower market value compared to Manuka honey. When 250 g jar of Manuka honey cost range from Rs. 5000.00 ($30) to Rs. 15000.00 ($96) Sri Lankan bee honey sold at around Rs. 600.00. The aims of this research work were to evaluate antibacterial activity and physicochemical characteristics of honey of different botanical sources, from different regions of Sri Lanka and compare those parameters with Manuka honey sample from New Zealand in order to understand the possible foreign market potential for Sri Lankan bee honey.

Materials and methods

Sample collection

Bee honey samples from different areas in Sri Lanka were collected during the honey season 2017 - 2018. These included bee honey samples from different regions of Sri Lanka (Ella, Elpitiya, Welimada, Loggaloya, Anuradhapura, Kothmale, Gampaha, Haputhale and Nuwara Eliya) and Manuka honey sample from Mosgiel, New Zealand.

Characterization of selected physicochemical properties of honey

Moisture content of bee honey was determined according to the method recommended by European Regional Standard for honey. Briefly brix values of the test samples, which were obtained from pre calibrated digital hand held pocket refractometer (PAL-3, Atago Instruments, Tokyo, Japan) at 20 °C were

converted into refractive indices. Then refractive indices were converted into percentage moisture content. Water activity (a_w) was determined using portable water activity meter (Ms -1, Novasina, Switzerland) at a temperature of 26 °C. To determine the pH, ten grams of honey was dissolved with 100 mL of CO_2 free distilled water in a 250 mL conical flask. Then pH of the solution was measured at ambient temperature using a pre calibrated digital pH meter (Starter 3000, OHAUS, USA). Total soluble solid (TSS) value was measured using digital hand held pocket refractometer (PAL-3, Atago Instruments, Tokyo, Japan). The specific gravity of honey sample at 27 °C was determined. 5-Hydroxymethylfurfural (HMF) content was measured using high-performance liquid chromatography [2]. For the preparation of mobile phase HPLC grade methanol: double distilled water; 10: 90 v/v ratios was mixed. Then 6 ppm, 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm HMF standard series were prepared using the standard stock solution by diluting with mobile phase. Five grams of bee honey sample was diluted up to 50 mL with the mobile phase. Then 20 µL of filtered solution was injected to the HPLC (SHIMADZU LC 20AT, Osaka, Japan), equipped with reversed phase column (Pinnacle 2, C18, 5 µm, 150×4.6 mm). Flow rate 1 ml/min and injected volume was 20 µL. HMF content was detected at 285 nm using UV-Visible detector (SHIMADZU, SPD-20A), and data was obtained through the C-R8A chromatographic recorder. The HMF content in bee honey samples was identified with comparing the standard HMF peak and it was quantified with the standard curve prepared accordingly. The colour of honey was measured using a Lovibond colour meter. The instrument was calibrated using the white slide provided. The L* indicates lightness Co-efficient. It ranges from 0 (black) to 100 (white). The a* and b* indicates the colour ranges from green (-a*) to red (+a*) and blue (-b*) to yellow (+b*).

Determination of antibacterial activity

Antibacterial activity was determined using agar well diffusion assay against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922) [3]. Briefly, uniform lawns were produced using sterile cotton swabs. A 5 mm sterilized borer was used to make wells in the nutrient agar plates and same amount of undiluted honey sample was introduced into each well. Sterile dH_2O served as a control. The plates were incubated at 37 °C for 24 h before visual assessment of the inhibition zones. The experiment was repeated three times. The quantification of microbial growth inhibition was determined by measuring the diameter of clear zones of microbial growth around the wells in the agar (including the well itself) using a Vernier caliper.

Pollen analysis

Honey sample was thoroughly mixed by vortex mixture. Then 10 g of honey sample was taken and it was dissolved in 20 mL of warm distilled water (40 °C). The mixture was centrifuged at 2500 rpm for 10 minutes. Then the supernatant liquid was removed using a dropper and the sediment was added again with

warm distilled water (40 °C) and centrifuged at the same rate. Supernatant liquid was removed and the sediment was obtained. Then a drop of sediment was spread over the glass slide and it was allowed to dry. Next it was microscopically observed in ×400 magnification under light microscope and pollen images were obtained. Then pollens were identified comparing to the reference pollen images. Absolute pollen count was obtained [2]. For quantification of the pollen types, at least 500 pollen grains were counted from each sample. The percentage frequency of the pollen taxa in all samples was calculated. The types of pollen were allocated to one of four frequency classes: predominant pollen types (>45% of the total pollen grains counted); Secondary pollen types (16%- 45%); important minor pollen types (3% -15%); and minor pollen types (<3%). The honey sample was characterized as unifloral, if it contained a predominant pollen type. Otherwise, it was considered as multifloral [4].

Statistical analysis

Data analysis was performed by IBM® SPSS® statistical software, version 20.0 for windows and results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used to compare the quantified variables in the samples of honey. The significance was calculated for $p < 0.05$.

Result and Discussion

Results revealed that both Sri Lankan bee honey and Manuka honey samples were within the acidic pH range (3.96-4.99). The low pH of honey would be inhibitory to many animal pathogens, with their optimum pH for growth in the range between 7.2 and 7.4 [5]. As shown in the Table 1, the mean moisture content (%) of all analyzed samples ranged from 17.00 % to 20.00 % and was in concordance with the maximum limit set by CODEX (20%). Due to the hyperosmotic nature of honey it would prevent the growth of bacteria and yeasts as it draws water out of the organism, killing them by desiccation. The water activity values of investigated samples varied from 0.549 to 0.586 and both of the honey water activity level were within the normal range (0.500-0.650). In this study, Anuradhapura honey sample had the lowest a_w. The low a_w does not support the growth of yeast and bacteria. The TSS values obtained in the present study varied between 77.37% and 80.26%, which suggests that the samples were most likely unadulterated. Both honey samples had acceptable level of specific gravity at (1.38-1.43) 27 °C (i.e. >1.35). Manuka honey had higher darkness (L*: 8.63) and it had lower yellowness (b*: 8.07) compared to Sri Lankan bee honey.

Table 1: Physiochemical parameters of honey samples

Sample	Loggaloya	Nuwaraeliya	Gampaha	Haputhale	Anuradhapura	Kothmale	Welimada	Ella	Elpitiya	Manuka
pH	4.99 ± 0.00*	4.63 ± 0.01	3.98 ± 0.01*	4.49 ± 0.01	4.03 ± 0.01*	4.41 ± 0.02*	4.86 ± 0.01*	3.96 ± 0.00*	4.62 ± 0.18	4.56 ± 0.01
Free acidity (meq/kg)	61.01 ± 0.84*	29.60 ± 0.26	64.73 ± 0.61*	30.40 ± 0.06	34.69 ± 1.58*	39.83 ± 0.54*	24.46 ± 0.51*	64.18 ± 1.36*	37.09 ± 0.75*	31.22 ± 2.84
Water activity	0.575 ± 0.00∗	0.578 ± 0.00∗	0.586 ± 0.00∗	0.582 ± 0.00∗	0.549 ± 0.00∗	0.579 ± 0.00∗	0.576 ± 0.00∗	0.578 ± 0.00∗	0.574 ± 0.00∗	0.555 ± 0.00
TSS (%)	78.60 ± 0.00*	77.73 ± 0.25*	77.37 ± 0.06*	77.47 ± 0.06*	79.03 ± 0.35*	78.63 ± 0.06*	77.60 ± 0.10*	77.87 ± 0.06*	78.47 ± 0.12*	80.27 ± 0.06
Moisture (%)	18.80 ± 0.00*	19.60 ± 0.20*	20.00 ± 0.00*	19.87 ± 0.12*	18.33 ± 0.31*	18.73 ± 0.12*	19.73 ± 0.12*	19.47 ± 0.12*	18.93 ± 0.12*	17.00 ± 0.00
Specific Gravity	1.41 ± 0.00*	1.41 ± 0.00*	1.38 ± 0.01*	1.40 ± 0.00*	1.41 ± 0.00*	1.41 ± 0.00*	1.40 ± 0.00*	1.40 ± 0.01*	1.41 ± 0.00*	1.43 ± 0.00
HMF (mg/kg)	0.00 ± 0.00*	98.33 ± 3.50*	0.00 ± 0.00*	82.20 ± 1.88*	127.94 ± 1.05*	0.00 ± 0.00*	138.83 ± 1.47*	104.39 ± 2.56*	77.57 ± 1.94*	91.52 ± 2.71

Results are expressed as mean values ± standard deviations. * Differences between manuka honey and tested Sri Lankan bee honeys were significant at the 0.05 level.

HMF is a toxic compound form in bee honey due to the high temperature. HMF content of Manuka honey (91.52 mg/kg) was higher than the CODEX, however average SLBH (62.92 mg/kg) values were up to the standard. Results revealed that regard to the physicochemical properties, Sri Lankan bee honey are in good quality.

The results revealed that Welimada, Haputhale, Nuwara Eliya honey samples and Manuka honey were contained Myrtaceae family predominantly. Loggaloya honey sample contain sapindaceae family predominently (frequency >45%). They were classified as unifloral honey. Families that occurred in more than 50% of honey samples included Myrtaceae (e.g Eucayptus sp.) (Found in 50% of the samples), Fabaceae (e.g Ehala) (70%), Asteraceae (e.g. Wild sunflower) (80%), Poaceae (e.g Vel maruk) (50%), Malvaceae (Pokuru wada) (70%). Among the collected bee honey samples there were 27% (Nuwara Eliya, Elpitiya, Kothmale) bee honey samples having pollen density >1,000,000/10g.

A variation in the antibacterial activity with floral source was observed. Unifloral and multifloral Sri Lankan bee honey samples were exhibited mean inhibition zone diameter for *S. aureus* 28.36 mm 29.17 mm and for *E.coli* 26.60 mm, 26.38 mm respectively. Mean bacterial growth inhibition zone diameter of Sri Lankan bee honey (SLBH) exhibited significantly higher antibacterial activity against *S. aureus* (28.94 mm) and similar antibacterial activity against *E. coli* (26.35 mm) compared to Manuka honey (*S. aureus* 26.69 mm, *E. coli* 25.39 mm). Therefore Sri Lankan bee honey has a potential to be sold at high or similar prices as Manuka honey.

Conclusions and Recommendations

Antibacterial activity of bee honey varies with the botanical origin. Sri Lankan bee honey used in this research has good physicochemical parameters and they have similar or higher antibacterial activity than Manuka honey. Therefore Sri Lankan bee honey has a potential to be sold at higher or similar prices as Manuka honey in the international market.

Further research needed to determine the methylglyoxal (MGO) content of Sri Lankan bee honey since MGO is the key chemical compound which contribute to the antibacterial activity of Manuka honey.

References

[1] P.C. Molan. “The Antibacterial Activity of Honey”. *Bee World*, vol.73, no.1, pp. 5-28, 1992.

[2] C. Jayasinghe, L. Ranganma, B. Jayakala, D. Karunasekara, R. Nandasiri, C. Jayasekara, H. Pakyanathan and S. Senarath. “Characterization and authentication of Sri Lankan bee honey”, *Proceedings of the International conference on Chemical Science, Institute of Chemistry, Sri Lanka*, 2012.

[3] D.N. Sheikh, S. Zaman, R. Naqvi. “Studies on the antimicrobial activity of honey”. *Pak J Pharm Sci* , vol. 8, no. 1, pp. 51-62, 1995.

[4] X.Y. Song, Y.F. Yao, W. De Yang. “Pollen Analysis of Natural Honeys from the Central Region of Shanxi, North China”. PLoS ONE, vol. 7, no.11, 2012. Internet: http://doi.org/10.1371/journal.pone.0049545 [June 28, 2018]

[5] C.M. Stewart. “Staphylococcus aureus and staphylococcal enterotoxins”. Ch 12 In: Hocking AD (ed.) *Foodborne microorganisms of public health significance.* 6th ed, Sydney, Australian Institute of Food Science and Technology (NSW Branch), 2003, pp. 359–380

DEVELOPMENT OF SYNBIOTIC YOGURT SUBSTITUTED WITH ELEPHANT FOOT YAM (*Amorphophallus paeoniifolius)* FLOUR

A.K.L.A.D. Madhushankhe* and S. Jayathilake

Department of Food Science and Technology, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP)-60170, Sri Lanka

**Corresponding author (email: dushan.post@gmail.com)*

Introduction

Interaction of dietary fiber with intestinal micro biota contribute many positive health related consequences in human. Specially, dietary fiber play major role in human health providing many health benefits [1]. The evidences showed that, the increasing consumption of dietary fiber lower the life time diseases [2]. Daily recommendation for dietary fiber for adults range from 18-38 g/d [3]. However, the world dietary fiber intake is below the recommended level [4].

Elephant Foot Yam (EFY) is a tuber crop that grown in mountain or hilly areas of subtropical countries, mainly in South East of Asia [5]. Although it has high production potential, still the EFY is known as underutilized crop. A most valuable component in the yam is Glucomannan.

Glucomannan is a water soluble dietary fiber that can be extracted from matrix of the EFY. Glucomannan content is about 5-9% of the yam [6]. The content of Glucomannan in the EFY flour is 51.3-96.9% [7]. The KGM is generally used as a soluble fiber and as soluble fiber it contributes prebiotic properties to the foods. Although the EFY is consumed as food in the world, the consumption is low in Sri Lanka and there is very less amount of product that used EFY as additive. Addition to that, utilization of the EFY that identified as underutilized crop in Sri Lanka is important.

Material and Method

Elephant foot yam were purchased from Kandy market. Kothmale Fresh milk (UHT) were purchased from Pannala Super market. Yogurt culture (50U) was purchased from Mawathagama, Kurunegala.

Preparation of crude konjac flour

The tuber was weighed, removing of epidermis, trimming of unnecessary particles and washing was done. Then it was sliced into pieces and slices are to be immersed in Sodium Metabisulphite solution. Then oven drying was done and continued until constant weight was reached. Grinding and sieving of dried slices were done.

Purification of crude konjac flour/Extraction of Konjac Glucomannan

Crude Konjac Flour (CKF) were stirred in ethanol at room temperature, followed by centrifugation to remove the aqueous ethanol. The resultant pellet was added

to centrifugation to remove the insoluble materials. Subsequently, rotary evaporation was performed to reduce the volume of the filtrate, followed by KGM precipitation with ethanol, vacuum filtration and freeze drying. The dried filtrate was ground and sieved to produce Konjac flour.

Evaluation of prebiotic activity

Prebiotic activity denotes the ability of a given substrate to support the growth of an organism relative to other organisms and relative to growth on a non-prebiotic substrate, such as glucose.

Prebiotic activity was evaluated as it (1) metabolized as well as glucose by probiotic strains and (2) are selectively metabolized by probiotics but not by other intestinal bacteria. It was performed by adding an overnight culture of each probiotic strain to separate tubes containing MRS Broth with glucose or prebiotic. The cultures were incubated under anaerobic conditions in an anaerobic chamber for Bifidobacterium and L. acidophilus strains. After 0, 24, 48h and 72 h of incubation, samples were enumerated on MRS agar.

Preparation of yogurt samples

Standardized milk was pasteurized and then the EFY flour, gelatin and sugar were added to milk and homogenization of mixture were done. The mixture was allowed to cool and then the yogurt culture was added and mixture was stirred. The mixture was poured into cups and sealing was done. The sample was incubated and was stored at refrigerated temperature.

Proximate Composition

Moisture content was analyzed by the method described in AOAC, 2005 [8]. The ash content was analyzed by the method as contain in AOAC, 2005 [8]. The Total Solids was determined by method as described in AOAC, 2005 [8]. Protein content was analyzed using Kjeldahl equipment. Fat content were analyzed by Soxtherm Fat Analyzer. Fiber analyzer was used to analyze the fiber content of the sample. The content of carbohydrate was determined by difference as described by AOAC, 2005 [8].

Physio-chemical Analysis

Centrifugal acceleration test were used to determine the syneresis rates of yogurts. The water holding capacity (WHC) was determined according to the method described by Bensmira and Jiang (2012). The titratable acidity was determined by the method described by AOAC, 2005 [8].

Microbial Analysis

Total plate count, Yeast and Mould count, Probiotic count were done for control and flour added yogurt samples.

Invitro Digestion

In vitro digestion was done according to the method described in Buriti *et al.,* 2010.

Sensory Analysis

Sensory evaluation were done by two different sensory panels. First panel was an untrained panel with 31 panelists and 2.5% and 5% flour added yogurt samples were tested. Second panel was a semi trained panel with 17 panelists. In here 2.5%, 3.75% and 5% of flour added yogurt samples were tested. Seven points hedonic scale was used for analysis. Appearance, color, texture, taste, aroma and overall acceptability were tested.

Statistical Analysis

The data obtained from proximate analysis were analyzed using SPSS16 software by analysis of variance (one way). Data values obtained from sensory evaluations were analyzed using SPSS16 software by nonparametric analysis.

Result and Discussion

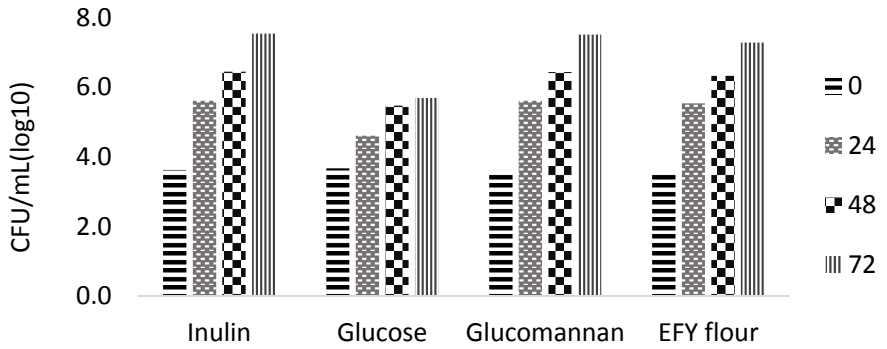


Figure 1. Prebiotic activity of Inulin, glucose, Glucomannan and EFY Flour at 0, 24h, 48h and 72h

Proximate Composition

Moisture content of the yogurt has decreased with the addition of flour. The flour not provide moisture itself to the yogurt. The moisture percentage get reduced due to increment of total solids content with the addition of flour. This may be the reason for the reduction of moisture percentage of yogurt sample with flour incorporation.

Ash content is increased with the incorporation of flour into yogurt. Elephant Foot Yam contains 3.98 - 4.78% of total ash [9] and EFY flour contains 2.99 ±0.20% of ash itself. That should be the reason for the enhancement of ash content in flour added yogurt sample.

The percentage of protein in the yogurt get reduced with addition of flour as the EFY contains low amount (1.12–1.63%) of protein [9].

Fat content has reduced with substitution of flour into the yogurt. The EFY normally known as a low fat tuber as it contains 0.105–0.141% of fat [9]. The flour only contains 0.62±0.03% fat itself and that should be the reason for lowering fat content with the incorporation of flour.

The fiber content of the yogurt sample has increased with addition of flour. Fiber plays a major role by providing synbiotic effect to the yogurt. Addition of flour into the yogurt has contributed significant amount of fiber. That may be the reason for increased in fiber content in flour added yogurt.

Carbohydrate content was measured by difference technique. The content of carbohydrate is increased with the addition of flour. High carbohydrate content (75.90±0.22%) in the flour should be the reason for increment of carbohydrates in the flour added yogurt.

Physico-chemical Analysis

Total Solids content in flour added yogurt sample is higher when compared to control sample. High content of total solids in the flour should be the reason for that.

Water holding capacity is denoted that the amount of whey separated from the yogurt matrix. Higher water holding capacity means lower amount of whey separation. The water holding capacity of flour added yogurt has reduced when compared with the control sample. High total solids content in the flour added yogurt may get precipitated by expelling serum due to centrifugal force. This might be the reason for reduction in water holding capacity with addition of flour into yogurt.

Syneresis rate means that the serum separation in the yogurt sample per unit weight of sample at unit time. High syneresis rate denoted that the high separation of serum in the sample. Since the flour added yogurt sample has high syneresis rate than the control sample, the serum separation of flour added sample is higher than the control sample. But, it is not significant. Serum separation can be increased when the pH of the sample is high. Because, the high pH cause breakdown of gel structure of the yogurt. At the same time high amount of particle-particle junctions in the gel structure cause shrinkage of the gel structure and release the serum.

Titratable acidity of the flour added yogurt is higher than the control sample while pH is lower in the flour added yogurt sample compared to the control sample. pH can be denoted as $-\log_{10}[H^+]$. Thus, increasing acidity in the yogurt sample cause reduction in pH value. That relationship can observed by comparing control and flour added yogurt sample. The pH value of the flour added sample has decreased

while increasing the acidity. The high fiber content in the flour added yogurt act as substrate (prebiotic) for probiotic bacteria. Since the activity of bacteria is increased, more lactic acid may results in the yogurt medium. This might be the reason for increasing total titratable acidity and reduction in pH of flour added yogurt sample compared to control.

Sensory Analysis

Yogurt samples were evaluated for appearance, color, texture, taste, aroma and overall acceptability. 2.5% of flour added sample was selected as highly acceptable sample.

Microbial Analysis

The total bacterial count has increased in the flour added yogurt sample when compared with control yogurt sample. Since fiber content in the flour added sample is higher than the control sample, it provides substrate and enhance the growth of bacteria. This might be the reason for the increment of bacterial count in the flour added yogurt. After two weeks storage, the bacterial count was ranged from $1‹10^6$ to $2‹10^6$. According to the literature, not less than $1‹10^5$ of viable cells/mL should be in products to use the term “probiotic”.

In-vitro Digestion

The probiotics counts were decreased when inoculated after digestion (Figure 1). Although the probiotic count was reduced in one log cycle, there was still enough amount of probiotics for colonization in the colon. Since the CFU were within the range from 10^6 to 10^7, the term ‘probiotic’ can be used for flour added yogurt sample.

The findings prove that EFY flour can successfully incorporated into yogurt providing synbiotic property to the product.

References

[1] Singh, R.K.; Chang, H.; Yan, D.; Lee, K.M.; Ucmak, D.; Wong, K.; Abrouk, M.; Farahnik, B.; Nakamura, M.; Zhu, T.H.(2017) Influence of diet on the gut microbiome and implications for human health. J.Transl. Med. 15, 73.

[2] Anderson, J.W., Baird, P., Richard, H.D.J., Ferreri, s., Knudtson, M., Koraym, A., Waters, V. and Williams, C.L.(2009)Health benefits of dietary fiber:*Nutritional review*.International Life Science Institute.67(4)p188-205.

[3] Institute Of Medicine (IOM) and U.S. National Academy of Science (2001) Dietery reference intake:*Proposed definition of Dietary Fiber.*Washington: D.C.National Academy Press.

[4] Jones, J.M. (2014) Dietary fiber intake,Disease prevention and Health promotion:an overview with emphasis on evidence from epidemiology.In : *Bioactive Carbohydrates for Food and Feed.* ed.1: Van de Kamp

J.W.,Netherland.Acedemic publishers:Asp.N.G.Miller jones,J.schaafsma, G.Wageningen.pp143-164.

[5] An, N.T., Dong, N.T., Le Dung, P. and Van Du, N. (2010) Characterization of glucomannan from some *Amorphophallus* species in Vietnam. Carbohydrate Polymers, 80(1): 308–311.

[6] Xiao, C.,Gao,S.,Li,G.andXang,Q.(1999)Preparation of Konjac Glucomannan and acrylamide grafted Konjac Glucomannan. *Wuhan University Journal of Natural Sciences*, 4(4): 459–46

[7] AOAC (2005) Association of Official and Analytical Chemists Washington DC (12th ed)

[8] Sreerag, R.S., Jayaprakas, CA. and Sajeev, M.S. (2014) Physico-chemical and textural changes in Elephant Foot Yam (Amorphophallus paeoniifolius) Tubers infested by the mealy bug, Rhizoecus Amorphophalli Betrem during storage. Journal of Postharvest Technology, 2: 177–187.

PINEAPPLE (*Ananas comosus* L.) PEEL WASTE BASED CELLULOSE FIBERS AS A PROBIOTIC ENCAPSULANT: CHARACTERIZATION

R.A.D. Rajapaksha*[1], N.M. Adassooriya[2], M.D.J.C. Sandarani[1], C.V.L. Jayasinghe[1]

[1]*Department of Food Science and Technology, Faculty of Livestock Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila, 60170, Sri Lanka*

[2]*Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, 10250, Sri Lanka*

**Corresponding author (email: arunikarajapaksha93@gmail.com)*

Introduction

The issue of food losses is a vital in the efforts to combat hunger, raise income and improve food security in the world's poorest countries. Agriculture is the most common livelihood of Sri Lankans, and almost eighty (80) different varieties of fruits and vegetables are grown in Sri Lanka. Sri Lanka produces around 710,000 metric tons of vegetables and around 540,000 metric tons of fruits annually [1]. The composition of the market waste in Sri Lanka as; vegetable wastes: 45%, fruit wastes: 35%, packing material: 15% and restaurant food waste: 3% [2]. Moreover, the post-harvest loss of fruits and vegetables is significantly high which is about, 30-40%. Therefore, damaged, immature and over ripened fruits and vegetables are commonly found in waste materials. Waste materials of fruit and vegetables include trimmings, peelings, seeds and whole fruits can be used to produce value added constituents.

Pineapple (*Ananas comosus* L.) is a seasonal, tropical and sub-tropical fruit. It is the fifth major cultivated fruit crop in Sri Lanka. Pineapple production of Sri Lanka in 2009 was 32,626 metric tons. During industrial processing, it generates 55% of waste from whole pineapple fruit including peel (40%), bagasse (23%), and stem and crown (14%).

Fruit residues in industrial sites pose environmental problems due to its high biodegradability, represents a loss of valuable biomass and an economic cost for companies. A high level of biological oxygen demand and chemical oxygen demand in pineapple wastes add to further difficulties in decaying [3]. Underutilized pineapple by products are good source of dietary fiber, phenolic compounds, minerals and antioxidants [4]. Preferably, they can be used in different food products in order to improve the textural and functional properties. Pineapple peel waste (PPW) can be used to isolate cellulose fibers. Cellulose is a natural polymer classified as homo-polysaccharide, which is widespread in nature, and it may be obtained from many natural sources, such as wood, cotton, seeds, vegetable and fruit biomass. Cellulose can be used to

probiotic encapsulation because it can increase the viability of probiotics. Encapsulation of probiotic cells with probiotic properties may have specific food and medical applications. The aims of this study were to evaluate the suitability of industrial pineapple peel waste as a source for isolation of cellulose fibers and to evaluate the feasibility of utilization of extracted fibers in probiotic encapsulation.

Materials and Methods

Materials

Pineapple peel waste (PPW) was obtained from Ceylon Biscuits Limited (CBL) Natural Foods Pvt LTD, Minuwangoda.

Probiotic (*Lactobacillus bulgaricus* and *Streptococcus thermophilus* mixer) culture was obtained from Morisons, Sri Lanka.

Bleaching (cellulose isolation)

Firstly, the fresh parts of the waste materials were separated and washed well. Then wet milling was done to reduce particle size. The prepared pineapple peel waste pulp was first washed with distilled water at 85 ^{o}C for 5min. Pulp was treated with an alkali (2% NaOH) at 80 ^{o}C for 2 h. Then pulp was filtered and washed with distilled water. The resulting residue was bleached with equal parts of sodium oxychloride (NaOCl) (2%, 5%, 7.5%, 10%) commercial bleaching powder (2%, 3%, 4%), acetic buffer (pH 4.5), and distilled water at 80 ^{o}C for 2 h. The product was washed several times with distilled water until the pH is neutral. Extracted fibers were dried at 45±5 ^{o}C until a constant weight is obtained [5]. The solid recoveries were calculated as yields. The produced cellulose fibers were characterized using Fourier transform infrared (FTIR) spectroscopy, powder X-ray diffraction (PXRD), and a scanning electron microscopy (SEM).

Acid Hydrolysis

Extracted cellulose fibers were dipped in 1M hydrochloric acid (HCl) for 3 h for acid hydrolysis. After that cellulose fibers were washed with distilled water until the pH is neutral.

Probiotic Encapsulation

After the activation of probiotic (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) culture in sterile distilled water probiotics were grown in MRS agar media at 37 ^{o}C for 36 h. Then, 10 g of extracted cellulose fibers were dissolved in 50 ml of distilled water. Prepared slurry was autoclaved at 121 ^{0}C for 20 min and allowed to cool. Probiotic culture was inoculated into sterile cellulose slurry. Sample was stirred for 4h to adhere the culture with fibers and incubated at 37 ^{0}C for 36 h. Finally incubated sample was freeze dried at -40 ^{0}C for around 30 h. Freeze dried samples were used for characterization.

Data Analysis

The solid recoveries were calculated as extraction yields according to the following equation:

$$Yield\% = W_1/W_0 \times 100$$

Where W_1 indicates the dry weight of the sample after the chemical treatment and W_0 indicates the initial dry weight of the residue [5]. Results were expressed as mean ± standard deviation.

The crystallinity index (CI) was calculated according to the following equation:

$$CI = (I_{002} - I_{am}) / I_{002} \times 100$$

where I_{002} is the intensity of the crystalline peak and I_{am} is the intensity of the amorphous peak (peaks at 2Θ of about 22 and 18, respectively) [5].

Results and Discussion

Table 1. Extraction yield calculations of Pineapple Peel Waste after bleaching step of the chemical pretreatment process.

Bleaching Compound	Mean Extraction Yield (Dry basis %)
2% bleaching powder	30.33±0.03
3% bleaching powder	28.29±0.02
4% bleaching powder	24.08±0.02
2% NaOCl	30.38±0.01
5% NaOCl	27.51±0.01
7.5% NaOCl	24.34±0.01
10% NaOCl	21.90±0.01

As shown in Table 1, several concentrations of two bleaching agents were used for the extraction of cellulose fibers. The majority of the material loss was seen in the alkali and bleaching steps of the process and the higher extraction yields were observed for 2% commercial bleaching powder and for 2% NaOCl, compared to higher concentrations. With the increment of the concentration of the bleaching agents extraction yield of cellulose from PPW is decreased due to the more removal of non cellulosic materials such as hemicellulose, lignin, pectin, tannin and betacarotene in the amorphous areas. It might be attributed repeated bleaching step required with higher concentrations for concentrations.

Based on the PXRD, the crystallinity index (CI) of isolated celluloses fibers was determined using PXRD diffractogram. The calculated CI for natural fibers extracted using 2%, 5%, 7.5% and 10% NaOCl from PPW were 34.78%, 54.77%, 57.17 and 43.04 respectively. CI of cellulose fibers extracted using 2%, 3%, and 4% bleaching powder were 47.71%, 48.60% and 46.89% respectively. These values are relatively lower value due to the presence of amorphous regions. Crystallinity index of cellulose was increased up to some extend (in case of NaOCl: 7.5% and in case of bleaching powder: 3%) with the increment of concentration

of bleaching agents due to dissolution of amorphous regions. Since the structural damages of the fibers, CI of cellulose extracted using 10% NaOCl and 4% commercial bleaching powder were reduced. PXRD analysis for dehydrated and acid treated cellulose extracted using 10% NaOCl gave the highest CI (55.96%) in acid treated sample than non-acid treated (43.04%) cellulose extracted using 10% NaOCl. The increase of the crystallinity upon acid hydrolysis indicated the dissolution of amorphous regions of the cellulose fiber. During the hydrolysis process, chemical acid penetrates and attacks the amorphous regions of the cellulose. CI of freeze dried cellulose were decreased compared to dehydrated samples. It may be caused by effects of low temperature. In general thermal annealing improve the crystallinity of the materials. Even the freeze dying causes the low temperature can facilitate the formation of amorphous regions [6]. Probiotic encapsulated fibers have given lower CI compared to samples without cultures due to the reduction of crystalline arrangements of the fibers; cultures are entrapped with the fibers and it caused to reduce the parallel arrangement of fibers. In comparison, CI of alkali treated PPW pulp (52.12%) was higher than CI of PPW pulp (12.63%). With the alkali solution the most of the non cellulosic compounds are removed. So, there is notable difference of CI in these samples.

SEM was carried out to analyze the surface morphology of the cellulose fibers. SEM images of cellulose fibers extracted using 2% commercial bleaching powder presented that cellulose is present as a three-dimensional structure with around 140 µm of diameter and elongated rigid micro fiber bundles which has nearly parallel arrangement. Fibrous structure of isolated cellulose using 10% NaOCl showed that there is no large agglomerations or rough surface and also reduced diameter of about 100 µm, due to high concentration of bleaching agent used. The surface of the cellulose fibers extracted using 10% NaOCl followed by acid treatment using 1M HCl was more smooth and more reduced diameter (around 80 µm) than non-acid treated cellulose fibers extracted using 10% NaOCl. Acids can dissolve more amorphous region in cellulose fibers. Probiotics can be seen in the surface of cellulose fibers extracted using 10% NaOCl. *Lactobacillus bulgaricus* presented as rod shape and *Streptococcus thermophillus* presented as cocci shape. Some of the bacteria are presented individually while others are presented as clusters arrangement (Figure 1).

The FTIR technique was carried out to study the functional groups present in cellulose (Figure 2). In the cellulose, the peaks are present at 3335 cm^{-1} and 1030 cm^{-1} can be assigned as O-H stretching and C-O stretching at C-6 respectively. The presence of glycosidic linkage is an evidence of the cellulose structure as these linkages bond anomeric carbon atom of saccharides to form polysaccharides. The alkali treatment of PPW pulp successfully showed the presence of a pure cellulose phase without lignin and hemicellulose.

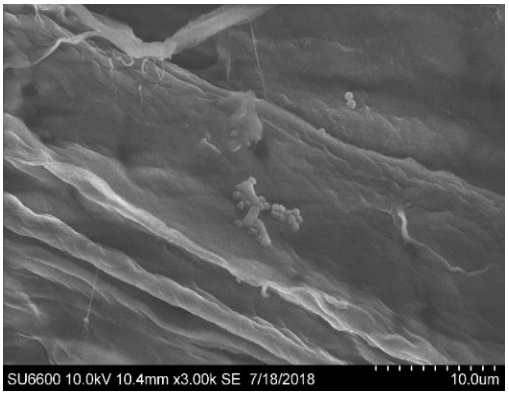


Figure 1. Probiotic encapsulated cellulose fibers extracted using 10% NaOCl.

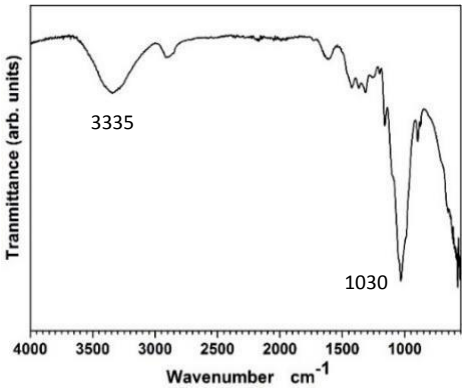


Figure 2. FTIR spectra of extracted natural cellulose fibers using 2% commercial bleaching powder from pineapple peel waste

Conclusions and Recommendations

In this study, cellulose fibers were successfully produced from the *Ananas comosus* L. peel waste by exposed to an alkaline treatment and a bleaching process. Cellulose fibers showed increased crystallinity, indicating the exposure of the crystalline phase after the removal of lignin and hemicellulose via pretreatments of PPW and the removal of the more amorphous region of cellulose via acid hydrolysis. SEM images showed significant changes on the surface morphologies of the fibers. The cellulose fiber surfaces became smoother and eventually showed reduction in size after acid hydrolysis. FTIR spectra of bleached fibers showed the typical functional groups of cellulose. Moreover, the feasibility of encapsulating probiotics in to cellulose fibers was demonstrated. This study indicated that there is a great production potential of cellulose from PPW which can be used in various industries.

This study suggests utilization of PPW in cellulose extraction and utilizing extracted cellulose in probiotic encapsulation. This will be more important if try to extract cellulose nanofibers to reduce the fiber diameter hence increase the surface area for higher attachments of probiotics. Acid hydrolysis using dilator bag and ball milling methods can be suggested for extraction of nano-cellulose. Also further research studies are essential to evaluate the viability and activity of the probiotics encapsulated in cellulose fibers and encapsulation percentage needed to be evaluated.

References

[1] Exports Development Board. (2013). "INDUSTRY CAPABILITY REPORT – SRI LANKAN FRUIT AND VEGETABLE SECTOR" Internet: www.srilankabusiness.com/pdf/fruit_and_vegetable.pdf [March.01,2018]

[2] M. Thenabadu, R. Abeyweera, J. jayasuriya and N. Senanayake "Anaerobic Digestion of Food and Market Waste; Waste characterisation and Bio-methane Potential: A Case study in Sri Lanka." *SRI LANKA ENERGY MANAGERS ASSOCIATION*, 23, p.29, 2014

[3] A. Upadhyay, J. Lama and S. Tawata "Utilization of pineapple waste: a review" Internet: https://www.nepjol.info/index.php/jfstn/article/view/8255,2010 [March.25,2018]

[4] R. Rodriguez, A. Jimenez, J. Fernández-Bolanos, R. Guillen and A. Heredia "Dietary fibre from vegetable products as source of functional ingredients" *Trends in food science & technology, 17*(1), pp.3-15,2016.

[5] L. Berglund, M. Noël, Y. Aitomäki, T. Öman and K. Oksman "Production potential of cellulose nanofibers from industrial residues: Efficiency and nanofiber characteristics" *Industrial Crops and Products*, *92*, pp.84-92, 2016.

[6] T. Einfalt, O. Planinšek and K. Hrovat "Methods of amorphization and investigation of the amorphous state" *Acta Pharmaceutica*, *63*(3), pp.305-334, 2013

PHYSICOCHEMICAL AND COOKING CHARACTERISTICS OF SELECTED SRI LANKAN TRADITIONAL AND IMPROVED RICE VARIETIES (*Oryza sativa* L.)

R.M.N.D.K. Rathnayake* and A.M.M.U. Adhikari

Department of Food Science and Technology, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), Sri Lanka
** Corresponding author (email: nishamini1993@gmail.com)*

Introduction

Rice (*Oryza sativa*) is one of a major cereal crop grown in the world having the third highest cereal production. Rice is consumed as a staple food in many countries including Sri Lanka. Rice has desirable physicochemical and cooking characteristics, which account for a wide distribution of this cereal around the world. Physical characters of rice are important in rice handling operations such as sorting, grading, threshing etc. Cooking characteristics of rice help in determination of consumer preference and acceptance and, chemical properties plays a major role in determination of nutritional value of rice as well as eating quality. Sri Lanka is one of the major rice producing countries in the world. Sri Lanka produces different rice varies including both traditional and improved cultivars. When considering Sri Lankan rice consumption; consumer and farmer preference towards traditional rice is drastically increasing, due to high nutritional value and medicinal usage of these rice cultivars. But very less scientific data is available and, very less amount of traditional varieties have been exploited in terms of evaluating the physicochemical and cooking properties of traditional rice varieties in Sri Lankan. Therefore this research was carried out to evaluate some selected physicochemical and cooking characteristics of eight underutilized traditional rice varieties, and four improved rice varieties grown in Sri Lanka in view of popularizing these varieties among the community and increasing interest of further research using these cultivars.

Materials and Methods

Sample collection

Eight traditional rice varieties known as Sudumurunga(SM), Hangimuththan(HM), Behethheenati(BH), Batapolal(BP), Kahawanu(KW), Kahamaala(KM), Kirinaaran(KN) and Ranthmbilial (RT) were purchased from local market Sri Lanka. Four improved rice varieties known as At 354, At 362, At 373, and Bg 406 were obtained from Rice research and development institutes in Sri Lanka. Then the paddy was de-husked and placed in airtight containers for future experiments. All the experiments were performed in triplicates from the prepared samples.

Determination of cooking characteristics

Minimum cooking time (MCT), volume expansion ratio (VER), water uptake ratio (WUR), percentage gruel solid loss (PGSL), and gelatinization temperature (GT) of rice varieties were determined as cooking characteristics.

For determination of MCT; 2 g of rice was cooked in 20 mL of water in a 50 mL beaker, and the time required to disappear the white core when pressing the kernels between two glass slides was taken as the MCT. For determination of WUR; 2 g of rice was cooked for the minimum cooking time and cooked rice weight was taken and the weight of cooked rice to raw rice was taken as the WUR. For determination of PGSL; 2 g of rice was cooked in 20 mL of distilled water and the cooking gruel was transferred to a pre dried and weighed moisture can and the gruel was oven dried overnight at 110 ^{0}C. After that the gruel weight was taken and the PGSL was calculated as weight of gruel solid per 100 g of raw rice weight [1].

For determination of VER; volume of 2 g of cooked rice and raw rice was taken using the water displacement method. Then ratio between cooked rice volume to raw rice volume was taken as the volume expansion ratio [2].

For determination of GT; six undamaged rice kernels were chosen and those were placed in a small petri dish. Then 2 mL of 1.7% KOH was added into petri dishes and the dishes were incubated in 30^{0}C for 23 hrs. The degree of kernel disintegration after 23 h was used to determine the GT [3].

Determination of physical characteristics

Length and width of 5 undamaged kernels were measured using a Vernier caliper. Grain size was determined according to the length of kernels and, the shape was determined according to the length to width ratio. Pericarp color was recorded by visual observation. [1][3].

For determination of gel consistency; 100 mg of rice flour was taken into a culture tube and treated with 0.2 mL of 95% ethanol and 2 mL of 0.2 N KOH and placed in a vigorously boiling water bath for 8 min. Then samples were removed from water bath and cooled in room temperature for 5 min and, again keep in an ice water bath for 15 min. After that tubes were horizontally laid over a graduated paper for 1 hour and, the length of the gel formed inside the tube was measured in millimeters [4].

Determination of chemical characteristics

Rice flour (particle size <0.425 mm) was used for chemical analysis. Moisture, ash, crude protein, crude fat, crude fiber, and carbohydrate percentages were determined according to the AOAC 2000 methods. Amylose content was determined according to the method proposed by Juliano in 1971 [5].

Statistical analysis

Statistical analysis was done using SPSS 16 software, using one way analysis of variance (ANOVA) and Turkey analysis.

Results and Discussion

The variety 'Sudumurunga' achieved cooking within shortest time (20.97 ± 0.66 min) whereas the variety 'Batapolal' required the longest time (51.04 ± 0.56 min) for cooking. When considering the mean minimum cooking time of traditional and improved varieties; except Batapolal, traditional varieties had a mean value of 28.66±6.46 min and improved varieties had a mean value of 38.90±8.87 min. Batapolal variety had an exceptionally higher value compared to other traditional varieties. However, when comparing the mean minimum cooking time, there was no significant difference ($P>0.05$) between traditional and improved varieties with respect to minimum cooking time.

Volume expansion ratio of the selected varieties were ranged between 0.833±0.00 and 1.51±0.29. Batapolal variety had the lowest volume expansion after cooking whereas Ranthambilial variety had the highest volume expansion after cooking. Selected traditional varieties had a mean volume expansion of 1.1254±0.15 and, selected improved varieties had a mean volume expansion of 1.1859±0.10. There was no significant difference ($P>0.05$) between the selected traditional and improved varieties in respect of volume expansion ratio.

When considering water uptake ratio; the lowest WUR was obtained by At 354 (1.0478±0.00) variety and, the highest water uptake ratio was obtained by Kirinaaran variety (2.0230± 0.00). Except Batapolal, the selected traditional varieties had a mean WUR of 1.7941±0.12 and, selected improved varieties had a mean WUR of 1.3921±0.25. Batapolal variety had an exceptional lower value compared to other traditional varieties. There was a significant difference ($P<0.05$) between traditional and improved varieties in respect of water uptake ratio.

The gruel solid loss percentage of the selected varieties were in the range of 3.27±0.01% to 10.06±0.00 %. The lowest gruel solid loss percentage was obtained by Hangimuththan and the highest was obtained by At 354 variety. There was a significant difference ($P<0.05$) between the selected varieties in relation to the percentage gruel solid loss. The mean PGSL for selected traditional varieties was 5.4573±1.83 % and the mean PGSL for selected improved varieties was 7.7306±2.57 %. There was a significant difference ($P<0.05$) between traditional and improved varieties with respect to percentage gruel solid loss during cooking.

Sudumurunga, Hangimuththan, Kahamaala, Kirinaaran and At 362 varieties had low GT s (55-69 ^{o}C) and, Kahawanu, Batapolal, Bg 406 and At 354 had intermediate GT s (70-74 ^{o}C). Behethheenati and At 373 had high-intermediate

gelatinization temperatures and,Ranthambilial had a high GT (>74 ^{o}C). However by the used method, the gelatinization temperature can be determined only as a range according to the results.

The physical properties of selected varieties are presented in the Table 1.

Table 1: Physical properties of selected Sri Lankan traditional and improved rice varieties

Variety	Color	Average length* /mm	Length class	Average width* / mm	L/W ratio	Shape	Gel Length*/ mm	GC class
RT	White	5.2±0.02	short	2.2±0.04	2.36	Medium	134±1.16	Soft
KN	White	4.8±0.02	short	2.1±0.02	2.29	Medium	98±1.00	Soft
KM	White	5.3±0.01	short	2.5±0.02	2.12	Medium	91±1.00	Soft
KW	White	3.9±0.02	short	2.8±0.01	1.4	Bold	107±1.00	Soft
HM	White	3.7±0.02	short	2.2±0.02	1.68	Bold	104±0.58	Soft
SM	White	5.6±0.03	short	2.3±0.03	2.43	Medium	132±0.58	Soft
BH	Red	4.1±0.03	short	2.3±0.02	1.78	Bold	125±1.53	Soft
BP	Red	5.5±0.02	short	2.3±0.03	2.39	Medium	129±1.53	Soft
At 354	White	6.6±0.03	short	2.4±0.02	2.75	Medium	49±1.00	Intermediate
At 362	Red	6.2±0.02	short	2.1±0.02	2.95	Medium	127±1.53	Soft
At 373	White	4.1±0.02	short	2.0±0.02	2.05	Medium	144±0.58	Soft
Bg 406	Red	5.7±0.24	short	2.9±0.03	2.04	Medium	103±1.16	Soft

*Mean ± SD

GC- Gel Consistency, SM – Sudumurunga, HM- Hangimuththan, BH- Beheth heenati, BP- Batapolal, KW- Kahawanu, KM- Kahamaala, KN- Kirinaaran, RT- Ranthambilial

When considering the physical properties, all the selected varieties were short in size. At 354 variety had an intermediate gel consistency whereas all other selected varieties had soft gel consistency.

Amylose content of the selected varieties were ranged between 18.43±0.15% and (25.52±0.44) %. There was a significant difference (P<0.05) between amylose contents of the selected varieties. The proximate composition for selected varieties are represented in the following table (Table 2).

Table 2: Nutritional properties of whole grains of selected Sri Lankan traditional and improved rice

Sample	Composition* %					
	Moisture	**Ash**	**Protein**	**Fat**	**Fiber**	**CHO**
SM	12.22 ± 0.27^{bcd}	1.51 ± 0.00^{b}	15.21 ± 0.02^{cde}	2.42 ± 0.06^{cde}	1.95 ± 0.69^{a}	66.69
HM	13.88 ± 0.35^{fg}	1.12 ± 0.25^{b}	15.24 ± 0.54^{cde}	3.60 ± 0.16^{f}	1.50 ± 0.91^{a}	64.66
BH	13.14 ± 0.42^{defg}	3.51 ± 0.59^{c}	14.58 ± 0.73^{cd}	3.27 ± 0.09^{f}	1.98 ± 0.25^{a}	63.52
BP	14.40 ± 0.26^{g}	1.20 ± 0.12^{b}	16.79 ± 0.23^{de}	1.95 ± 0.36^{bc}	2.11 ± 0.99^{a}	63.55
KW	14.02 ± 0.13^{g}	0.44 ± 0.00^{a}	17.08 ± 0.88^{e}	1.49 ± 0.05^{ab}	0.62 ± 0.14^{a}	66.35
KM	13.68 ± 0.57^{efg}	1.37 ± 0.01^{b}	13.77 ± 056^{bc}	1.03 ± 0.14^{a}	1.44 ± 0.08^{a}	68.71
KN	12.54 ± 0.30^{cdef}	1.27 ± 0.11^{b}	16.50 ± 0.28^{de}	2.36 ± 0.03^{cde}	1.33 ± 0.16^{a}	66.00
RT	12.36 ±0.10^{bcde}	1.00 ± 0.06^{ab}	11.26 ± 0.61^{a}	1.19 ± 0.20^{a}	1.89 ± 0.60^{a}	72.30
At 354	8.92± 1.19^{a}	1.15 ± 0.00^{b}	13.05 ± 0.84^{abc}	2.12 ± 0.05^{cd}	1.96 ± 0.68^{a}	72.80
At 362	11.25 ± 0.11^{cdef}	1.24 ± 0.02^{b}	12.10 ± 0.28^{ab}	2.77 ± 0.18^{e}	1.78 ± 0.46^{a}	70.86
At 373	11.11 ± 0.14^{bc}	1.59 ± 0.00^{b}	17.11 ± 0.76^{e}	2.59 ± 0.00^{de}	0.98 ± 0.40^{a}	66.62
Bg 406	12.54 ± 0.37^{b}	1.11 ± 0.01^{b}	13.67 ± 0.79^{bc}	2.65 ± 0.19^{e}	5.90 ± 0.06^{b}	64.13

*Mean ± SD, Means that do not share a letter within the same column are significantly different (P<0.05

SM – Sudumurunga, HM- Hangimuththan, BH- Beheth heenati, BP- Batapolal, KW- Kahawanu, KM- Kahamaala, KN- Kirinaaran, RT- Ranthambilial

The physical properties of rice are important in determining consumer preference and market value of rice. Specially the grain appearance influences the buying behavior of customers. In addition to that, physical properties are important in designing machineries for rice processing operations such as sorting, grading, milling etc. Also these properties help in harvesting of grains, transporting and dimensioning of storage spaces. Chemical properties of rice are mainly important in determining nutritional value of rice. Apart from that, knowing nutritional properties helps to use these varieties in new product development. Cooking quality parameters are important to determine consumer preference and acceptance. Different consumers need different cooking characteristics of rice. Therefore knowing the cooking characteristics help in selecting suitable rice cultivars for commercialization and use in breeding programmes.

Conclusions and Recommendations

Traditional rice varieties investigated here have desirable physicochemical and cooking characteristics. Therefore there is a good potential to popularize these varieties among the community. Also the results obtained herein can be used for rice variety improvement programmes and in selecting suitable traditional rice varieties for commercial cultivation.

References

[15]H.A.P.W. Hettiarachchi,S.P. Rebeira,B.D.R. Prasantha, and H.A.M. Wickramasinghe, "Diversity of physical and cooking quality characters of selected traditional and improved rice varieties in Sri Lanka", *Sri Lankan Journal of biology*,1(1)., pp 15-26, 2016

[16]A P. Bentota, , D.Rajapakse, N. Ediriweera," Cooking and Eating Quality Traits of Some Sri Lankan Traditional Rice Varieties across Yala and Maha Seasons" , *Tropical Agriculture Research*, 20, pp 168-176, 2008

[17]N. D. Cruz and G.S. Khush, "Rice Grain QualityEvaluation Procedures" in Aromatic Rices,1st edition,1,R.K. Singh, U.S. Singh, G.S. Khush, Ed. New Delhi: Mohan Primlani for Oxford & IBH Publishing Co. Pvt. Ltd., 2000, pp 15-29

[18]G. B. Cagampang, C.M. Perez, B.O. Juliano, "A Gel Consistency Test for Eating Quality of rice", *Journal of science, Food and Agriculture*, 24, pp 1589-1594, 1973

[19]B.O Juliano," A simplified assay for milled rice amylose", *Cereal Science Today*, 16(11), pp 334-340, 1971

VALUE RELEVANCE OF INTEGRATED REPORTING: A QUALITATIVE ANALYSIS

P.M.D.S. Pathiraja* and W.A.N. Priyadarshanie

Wayamba University of Sri Lanka
**Corresponding author (email: dhananjayapathiraja01@gmail.com)*

Introduction

Integrated Reporting (IR) is a newly emerged concept in financial reporting arena and organizations tend to adopt IR for their reporting aspects. When considering the Accounting arena, the reporting dimensions have been gradually evolved and it is still transforming for new aspects which gives superior disclosures. During last decades, stakeholders primarily expect not only financial information but also non-financial information. For aid of this requirement the sustainability reporting has come out and it facilitated a separate report for demonstrating non-financial performance of the entity. Despite availability of information, many stakeholders were unable to use pertinently the disclosed information due to separation of reports. This is the point where the concept of integrated reporting is emerged so as to depict the holistic view of the entity.

Integrated Reporting is still an immature concept in Sri Lanka. Adoption of integrated reporting is not yet compulsory for the companies listed in Colombo Stock Exchange (CSE) and companies voluntary adopt IR. Many researchers have proved that IR adds value to the company. Lee et al., (2015) have investigated the association between Integrated Reporting and firm Valuation. The sample was the listed firms in South Africa. This study has explored the association between cross-sectional variation in Integrated Reporting disclosures and firm valuation in the period after the implementation of Integrated Reporting. As the conclusion it suggests that the firm valuation is positively associated with Integrated Reporting disclosures. Study revealed that the benefits of Integrated Reporting exceed its costs. Martinez (2016) has investigated the effects of Integrated Reporting on the firm's Value by considering the voluntary adopters of the IIRC's framework. The purpose of this paper was to evaluate potential external benefits related to capital markets of the IR Framework on a sample of international voluntary adopters. The selected data sample is comprised exclusively of firms included in the IIRC's database as of September 2016. The results indicate IR is positively associated with market value and expected future cash flows, but not with bid-ask spread or implicit cost of capital.

Thus, IR can be depicted as a value creation momentum as proved by the previous studies. Companies in Sri Lanka also currently examining this new concept and

trying to adopt IR for their reporting dimensions. Some companies have already adopted IR for their reporting perspectives. But it should necessarily be examined whether companies adopt IR with better understanding of its value relevance or blindly they adopt it as a trend. If a new change is adopt without proper understanding, then it will not provide expected results. So knowing the concept of IR and its value relevance are highly required by the management of the company. So this study focused to investigate the understanding of IR concept and its value relevance by the management of the company. The purpose of this analysis is to evaluate the companies' managers and executive's perception relating to the value relevance of IR for their entities. Many researchers focused to examine the basis of IR concept, benefits of adopting IR, value relevance of this concept and its association with firms characteristics. But understanding of this concept among practitioners are rarely discussed and this study tries to fill that empirical gap.

Materials and Methods

This study is a qualitative study. Data was collected through online questionnaire. Firstly, authors found email addresses of key accounting personnel of IR adopted companies in Sri Lanka. Then 28 email addresses could be found and online survey was sent to those 28 companies key accounting personnel through emails. But only 13 companies have responded to the survey. Discussion of this study is based on the views of that 13 responders.

Results and Discussion

When examining the responses it was clear that all responders clearly identified the reason for adopting IR. All they know that the IR add value to the company. Senior Manager (Finance) of a large banking institution mentioned the following comment to prove this point;

"IR helps entities to improve its internal processes through integrated thinking which in turn reinforces the value creation process."

Senior Accountant of one of large diversified companies has a similar view;

"We adopt IR for our reporting, because it is the best way to show company's holistic view and it adds value to the entity by combining financial and non-financial performance accompanying with business value creation model"

So it is clear that companies adopt IR with proper knowledge of its value relevance and not blindly.

Responders have perceived benefit of IR in various extents. 85% of responders see the benefit of IR as better understanding of the relation between financial and non-financial performance. All responders indicate that IR helps to attract long term investors. 77% responders identified that IR improved internal measurement and control systems for producing reliable and timely non-financial information. Two responders state that lower reputational risk is also an advantage of IR. So these all advantages were considered as benefits of IR by two responders only. It is clear that most adopters know that IR assist with demonstrating financial information as well as non-financial information. Further, responses revealed that mostly achieved benefit among above mention benefits is better understanding of the relation between financial and non-financial performance and attracting long term investors.

Survey test the participants' perception regarding the effect of IR adoption on increment of share price, revenue and total assets. All responders state IR adoption results in to increase of share price. Head of Finance of a manufacturing conglomerate stated that;

"Integrated Reports are mostly referred by stakeholders of the company and they easily grab the view of company with value addition process. Attracting more stakeholders to the company means demand to its shares also increase. Thus prices are also automatically increased"

Reflecting a similar situation Hughen et al., (2014) highlighted that adoption of IR result in to increase of share prices of the entity. Researchers of this study mainly focus toward the improving stakeholder value through IR adoption. Amir and Lev (1996) confirms that non-financial information has a significant relation to stock price and its value-relevance. Many organizations find that financial reporting alone no longer satisfies the needs of shareholders, customers, communities, and other stakeholders for information about overall organizational performance. Combination of all information gives superior view of the organization. When reports are in higher level of assurance for depicting entity's view, more stakeholders demand for its shares. So this phenomenon result in to boost the share prices of the company.53% responses indicate that IR affect to increase asset of the company. 38% response depicts that revenue will boost due to the adoption of IR.

Survey last question was created to get an idea about the process of measuring or reviewing the level of IR in organizations. 62% responders indicate that they periodically investigate the adoption of IR & it's perceived value to the company. 76% of responses mention that company conduct surveys to get stakeholders' opinion in relation to value addition due to the adoption of IR. None of the

responders' companies use index to measure the level of disclosure of IR in their reporting. But few responses highlighted that they view the value addition of IR through their business value creation model. It is common that many IR adopters depict the value addition of IR by using their business model. This is more easy to communicate value rather explaining by using paragraphs. Whenever a business enterprise is established, it either explicitly or implicitly employs a particular business model that describes the design or architecture of the value creation, delivery, and capture mechanisms it employs (Teece, 2010). The essence of a business model is in defining the manner by which the enterprise delivers value to customers, entices customers to pay for value, and converts those payments to profit (Teece, 2010)

Conclusions and Recommendations

This study is consisted with pure qualitative analysis with analyzing the views of key personnel in accounting and finance sections of 13 companies listed in CSE. It is revealed that most companies adopted IR with better understanding of its value relevance. More people view that IR adoption helps to boost share prices. So it can be concluded that, companies in Sri Lanka tend to adopt IR after identifying its value addition and to gain more benefits to the entities.

Above qualitative study was done by considering small sample of data. And also finding can be generalized only to Sri Lankan companies. Further, the responders of this survey can be any personnel relating to accounting and finance field. So sometimes participants may not be key personnel directly involve to IR adoption. And also each responder's view represents company's perspectives regarding IR. But this study depicts the real perception of personnel rather analyzing numerical data. So this study will assist to get a natural view of adoption of IR.

Since IR adoption is not yet compulsory in Sri Lanka, companies adopt IR in various levels or degrees. Sometimes companies may not disclose major elements of IR. But they mention that they have generated an integrated report. Then there will be a quality issue in that report. So companies should measure the level of IR adoption in their companies. Then they can get an understand about the degree of IR adoption. Responders have viewed that they use various methods for measure the adoption of IR. But none of them use an index for measuring the level of IR adoption. If an entity use an index, then it will possible to gain a proper understanding about the degree of IR adoption. And also companies will able to compare the quality of integrated report. For an example, if a company annually measure the level of adoption of IR by using an index, then the relative progress

of adopting IR can be compared with previous years. So it is highly recommended companies to use an index to measure the level of IR adoption.

References

[1] K.W. Lee, G. Hian and H. Yeo, "The Association between Integrated Reporting and Firm Valuation," *Review of Quantitative Finance and Accounting, forthcoming,* vol. 4, no. 1, p. 47, 2015.

[2] C. Martinez, "Effects of IntegrEffects of Integrated Reporting on the Firm's Value: Evidence from Voluntary Adopters of the IIRC's Framework," 2016.

[3] L. Hughen, A. Lulseged and D. R. Upton,"Improving Stakeholder Value through Sustainability and Integrated Reporting," *The CPA Journal,* vol. 84, no. 3, pp. 57-61, 2014.

[4] E. Amir and B. Lev, "Value-relevance of non-financial information: the wireless communication," *Journal of Accounting and Economics,* vol. 22, no. 3, pp. 3-30, 1996.

[5] D. J. Teece, "Business Models, Business Strategy and Innovation," *Long Range Planning,* vol. 43, no. 2-3, pp. 172-194, 2010.

ASSOCITAION OF SELECTED INFLAMMATORY MARKERS IN ACUTE AND CHRONIC LOWER BACK PAIN IN LUMBAR DISC HERNIATION

N.D. Withanage*[1], H. Peiris[2], S. Perera[3], L.V. Athiththan[2]

[1]*Department of Allied Health Sciences, Faculty of Medical Sciences, University of Sri Jayewardenepura*
[2]*Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura*
[3]*The Central Hospital, Colombo 8*
**Corresponding author (email: withanagend@sjp.ac.lk)*

Introduction

Lower back pain (LBP) is a significant health problem that affects majority of the people worldwide. It has been documented that pain associated with back and neck is rated as the second commonest disorder, where respiratory tract infections is rated as the most common. According to available data 70 to 85 % people has suffered from LBP at any time of their life, making this as a major problem in health care services [1]. Importantly, LBP is considered as the main single cause for absence of work place and it also account for 12.5 % sick leaves among employees in the world. Although there are numerous causes for LBP, lumbar disc herniation (LDH) is considered as the major determinant of LBP [2]. However, evidences suggest that some patients do not have symptoms of radicular pain even with large with herniations, on contrast, another group of patients' exhibit severe radicular pain without disc herniation. Therefore, direct compressions on nerve roots partially explain the pathophysiology of LBP. Patients having LBP on most days of the week for at least for three months duration were regarded as subjects with chronic lower back pain (CLBP), whereas patients with LBP less than three months duration were considered as acute lower back pain (ALBP) subjects. Recent studies have suggested that herniated disc tissue causes an inflammation and irritation around the nerve roots by secreting pro-inflammatory markers such as interleukins, cytokines and nitric oxide. Irritations around nerve roots initiate the pain [3]. It was documented that cytokines particularly interleukin-6 (ILs) could give rise to the elevation of acute phase proteins; C-reactive protein (CRP), high sensitivity C-reactive protein (hs-CRP) and E-selectin. Studies have found increased hs-CRP, interleukins (IL-1, IL-6 and IL-8) and tumor necrosis factor-α (TNF-α) in patients with LBP. However, little information is known about the predictors of CLBP and ALBP. Therefore, the present study was conducted to investigate the association of some selected inflammatory markers such as hs-CRP, CRP and E-selectin with ALBP and CLBP.

Materials and Methods

Study design and setting

A case-control study was carried out in the Central hospital and Faculty of Medical Sciences, University of Sri Jayewardenepura after obtaining written consent from all participants. The study was approved (29/14) by the Ethics Review Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura.

Study subjects

Test: Subjects (n = 104) who had back pain and confirmed for lumbar disc herniation with Magnetic Resonance Image (MRI) by a consultant neurosurgeon and consultant radiologist.

Control: Individuals (n=104) without back pain at least for the previous one month period of the study.

Sample collection

Venous blood was taken from all the participants adhering to standard protocols for phlebotomy. It was allowed to clot for 45-60 minutes at room temperature and serum was separated by centrifugation at 3000 rpm for 5 minutes. Aliquoted serum sample of 100 µL was taken for hs-CRP, CRP and E-selectin analysis.

Laboratory assessment methods

KONE 20XT auto clinical analyser was used for the estimation of hs-CRP and CRP, while solid phase enzyme linked immunosorbent assay (ELISA)(Sigma Aldrich Corporation, USA), was used for the determination of serum E-selectin levels of the individuals. For the E-selectin, absorbance was measured at 450 nm. E-selectin levels were estimated against a standard curve. Quality control (QC) for both investigations was performed by the QC reagents supplied by the manufacturers.

Statistical data analysis

Non-parametric tests were used for statistical analysis using SPSS version 20.0. A p value ≤ 0.05 was considered statistically significant.

Results and Discussion

Among the test subjects majority presented with CLBP (81.7 %) while 18.4 % had ALBP. Mean age for test subjects with ALBP and CLBP was 41.6 ± 16.2and 44.1 ± 15.8 years respectively, while mean age for controls was 43.2 ± 15.2 years.

Results showed that there is no significant difference in hs-CRP, CRP and E-selectin levels among ALBP and CLBP patients (p=0.27, p=0.86 and p=0.83). However, CLBP patients had elevated levels of hs-CRP,CRP and E-selectin (1.76 mg/L, 4.30 mg/L and 9.40 ng/mL) compared to those of ALBP (hs-CRP=1.13 mg/L, CRP=4.20 mg/L and 7.0 ng/mL) (Table 1). However, when compared to controls both ALBP and CLBP patients had significantly elevated hs-CRP and CRP levels

(p=0.000 and p=0.001. There is no significant difference in serum E-selectin level in CLBP and ALBP patients when compared to controls (Table 2).

Table 1. Concentration of selected inflammatory markers in patients with acute back pain and chronic back pain

[a]Results of Mann-Whitney *U* test;hs-CRP, CRP and E-selectin

Table 2. Concentration of selected inflammatory markers in patients with acute back pain and chronic back pain compared to controls

Inflammatory markers	ALBP (Median)	CLBP (Median)	*U*[a]	*Z*	P value
hs-CRP (mg/L)	1.13	1.76	676.0	-1.11	0.27
CRP (mg/L)	4.20	4.30	786.0	-0.18	0.86
E-selectin (ng/mL)	7.0	9.40	782.5	-0.21	0.83

Inflammatory markers	ALBP (Median)	CLBP (Median)	Control (Median)	Chi square[b]	p value
hs-CRP (mg/L)	1.13	1.76	0.88	16.10	0.000
CRP (mg/L)	4.20	4.30	3.00	14.29	0.001
E-selectin (ng/mL)	7.0	9.4	13.3	0.20	0.990

[b]Results of Kruskal-Wallis test; hs-CRP, CRP and E-selectin levels

Studies have reported increased hs-CRP and CRP in patients with LDH when compared to controls. Although in the present study, mean hs-CRP concentrations in test group (ALBP and CLBP) were elevated than the normal reference range, in some studies elevated CRP and hs-CRP in LDH patients remains within the normal reference range [4]. Though the present study has found increased hs-CRP and CRP more prominent in CLBP, where some studies have found elevated inflammatory markers in ALBP patients [4, 5]. Some studies have contradictory findings suggesting that hs-CRP in CLBP remain constant with no correlation to the severity of pain [1]. Similar to the present study findings on E-selectin, another reported study also stated that E-selectin levels did not show association between severities of pain or pain related functions in LDH. Although, we have not evaluated modic changes associated with LDH, the elevation of hs-CRP and CRP in CLBP in the present study suggests that inflammatory phenomenon between the vertebral end plates followed by herniated intervertebral disc may be the reason for elevation of those inflammatory markers.

Conclusions and Recommendations

Present study confirms that inflammatory markers hs-CRP and CRP as sensitive markers to assess and evaluate the CLBP and ALBP associated with LDH. Further, the present study strongly recommends prescribing anti-inflammatory

treatments in patients with elevated inflammatory markers in order to control the local inflammation irrespective of the severity of LBP.

References

[1] Gebhardt K, Brenner H, Stürmer T, Raum E, Richter W, Schiltenwolf M, et al. The course of high-sensitive C-reactive protein in correlation with pain and clinical function in patients with acute lumbosciatic pain and chronic low back pain—A 6 months prospective longitudinal study. European Journal of Pain. 2006;10(8):711-.

[2] Manek NJ, MacGregor A. Epidemiology of back disorders: prevalence, risk factors, and prognosis. Current opinion in rheumatology. 2005;17(2):134-40.

[3] Park CH, Lee SH. Investigation of high-sensitivity C-reactive protein and erythrocyte sedimentation rate in low back pain patients. The Korean journal of pain. 2010;23(2):147-50.

[4] Sugimori K, Kawaguchi Y, Morita M, Kitajima I, Kimura T. High-sensitivity analysis of serum C-reactive protein in young patients with lumbar disc herniation. The Journal of bone and joint surgery British volume. 2003;85(8):1151-4.

[5] Le LG, Borderie D, Kaplan G, Berenbaum F. Systemic inflammatory response with plasma C-reactive protein elevation in disk-related lumbosciatic syndrome. Joint, bone, spine: revue du rhumatisme. 2000;67(5):452-5.

Acknowledgement

University Grants Commission (Grant no-VC/DRIC/PG/2013/SJP/02) and University of Sri Jayewardenepura research grant (ASP/01/RE/MED/2016/43) for the financial assistance.

PREVALENCE OF NOMOPHOBIA AND ITS EFFECT ON PSYCHOLOGICAL WELLBEING IN SMARTPHONE USING UNDERGRADUATES OF A SELECTED MEDICAL FACULTY IN SRI LANKA

V.J. Meegoda*, M.M.Y.N.B. Mapa, A.S. Matharage

Faculty of Medicine, University of Colombo
**Corresponding author (email: jithmalmeegoda@gmail.com)*

Introduction:
Telecommunication is one of the rapidly spreading media on the planet, encouraging an emergent "mobile culture" specially among the younger generation (Kanmani et al, 2017). According to the Telecommunication Regulatory Commission of Sri Lanka, country's mobile market is relatively small but mature and expanding compared to the neighboring South Asian countries (Annual report 2017 Q3). Most impressively, Smartphones introduced the 'World of Apps' where applications allow the user to do almost anything and everything and thus shift a part of our work to e-devices. A new disorder termed Nomophobia (NMP) (a portmanteau for NO MObile phone- PHOBIA) has garnered attention from researchers. Nomophobia is a disorder of contemporary digital and virtual society and refers to "discomfort, anxiety, nervousness, or anguish caused by being out of contact with a mobile phone, internet or its services" (King et al, 2013; Yildirim et al, 2015). It has been proposed to be included in the next version of Diagnostic and Statistical Manual of Mental Disorders (Yildirim et al, 2015).

The term was coined during a 2008 study by the United Kingdom (UK) Post Office who commissioned "YouGov", a UK-based research organization that sampled 2163 people to look at anxieties suffered by mobile phone users. Yildirim has been the pioneer in developing a tool to assess nomophobia. In 2015 through a two phase study he was able to identify the four themes of nomophobia (not being able to communicate, losing connectedness, not being able to access information and giving up convenience) upon which the validated questionnaire is based on.

Justification
In the Sri Lankan setting, young adults (age group 20-24) are the main contributors to the ever expanding smartphone market (Department of census and statistics 2016). Smartphones have become a must have equipment for medical undergraduates to access the vast array of information through internet and other applications (Kanmani et al, 2017). Numerous studies have shown that majority of medical students are nomophobic or at risk of developing nomophobia (Pavithra et al 2015, Sharma et al 2015). Thus, our selection of medical undergraduates for this research as they are expected to go through a tight schedule and has a higher possibility of feeling disconnected to their

smartphone and its services for the most part of the day. The emerging concept of nomophobia has not being addressed in the Sri Lankan setting to any extent. Many of the western countries and India had paid attention to the prevalence of nomophobia, its effects, determining factors and methods to reduce its adverse effects. Faculty of Medicine, Colombo was particularly chosen for the study as the area provides satisfactory telecommunication infrastructure thus allowing full access to the capabilities of smartphones. Therefore, a convenient and a large sample could be drawn from the population.

Objectives

To assess the usage patterns of mobile devices, prevalence of nomophobia and its effects on psychological wellbeing in smartphone using medical undergraduates.

Materials and Methods

A cross sectional analytical study was carried out at the Faculty of Medicine, University of Colombo (FoM,UoC). Out of the six batches, students of five batches were randomly selected for the study. Undergraduates registered at the FoM,UoC undergoing the medical curriculum, who were currently using a smartphone were included in the study. Foreign and final year (2011 A/L) students were excluded. 150 students were selected randomly, 30 from each of the five batches. Only the students who have given their written informed consent after reading the information sheet and clarifying any doubts with the principal investigators were included in the study.

A self-administered questionnaire was developed based on the objectives of the research. There was no need for it to be translated into other languages since the medical curriculum is conducted in English.

The questionnaire consisted of four parts. First part was focused on basic socio-demographic details. Second part collected data on patterns of smartphone usage, third part assessed the nomophobic status through the validated NMP-Q (Nomophobia Questionnaire) developed by Yildrim et al (2015), which has been validated in many countries including Turkey (Gezgin,2017), India (Prasad et al,2017; Dasgupta et al,2017; Sharma et al,2017; Sharma et al,2015;Pavitra et al,2015; Kanmani et al, 2015), Pakistan (Nawaz et al,2017) and Nigeria (Okoye et al,2017) through multiple researches. Fourth part assessed some aspects of psychological well-being among the participants.

The prevalence of nomophobia was established using the validated nomophobia questionnaire (NMP-Q). The answers to each question were summed up and a score of ≤20 was taken as no nomophobia, 21- 59 as mild nomophobia, 60- 99 as moderate nomophobia and 100- 140 as severe nomophobia as given in the manual of the validated questionnaire. The variables were analyzed in

percentages and their association with nomophobia was established. The significance of each socio-demographic/smartphone usage parameter for development of nomophobia and its effect on psychological well-being was proven via the Chi-Square test, taking the p-value as <0.05. The data were analyzed using SPSS software version 24 (Statistical Package for the Social Sciences).The ethical considerations were reviewed by the Ethics Review Committee of FoM,UoC and permission was obtained from Dean of the faculty prior to commencing the research.

Results and Discussion

A total of 142 completed responses were received with a cumulative response rate of 94.67%. Major reason for acquiring a smartphone was to use in case of emergency (26.1%) and main use was identified as voice calls (34.6%). Majority of the participants (38%) accessed internet for browsing social media while 13.4% of the participants had selected academic purposes as their main reason for accessing internet via smartphone. The mean time the study population spent on smartphone was 3 hours/day and spends a mean value of Rs.799.65 on their smartphone on a monthly basis.

Out of the study population, 93.7% checked their smartphones during clinicals/ lectures. According to a cross sectional study conducted by Prasad et al (2017) in dental students in India, 24.7% agreed that they frequently check their smartphone during classes or during clinicals. The difference in percentages could be due to the disparity in cultural, social and economic aspects of the two communities. A major proportion of students (95.8%) thought that their smartphone saves them time because it serves as a rapid medium of information access, while 80.3% of the students thought that they spend too much time on their smartphone which they could have otherwise used for studies. Out of them 65.5% had tried to reduce their use while only 34.4% had been successful. In a study conducted by Pavithra et al (2015) to assess nomophobia among medical students in Bangalore a similar situation was identified.

Prevalence of nomophobia in the study population was assessed using the validated nomophobia questionnaire where it was found out that 100% of the study population was nomophobic, which was an unseen phenomenon in any of the previous studies conducted in foreign countries. According to Sharma et al (2015) 73% of the 3rd year medical students in North India were nomophobic while only 39.5% of the medical students in Bangalore were nomophobic according to Pavithra et al (2015). According to Dasguptha et al (2017) comparing medical and engineering students, 45.4% of the medical students were found out to be nomophobic. The remarkably high percentage of nomophobia in our study could be due to the high sensitivity of the NMP-Q (nomophobia questionnaire). Limited sample size and socio demographic and cultural differences may have contributed to this unusually high result. Out of the study participants, 28.2% had

mild nomophobia, 62.0% moderate nomophobia and the remaining 9.9% were revealed to have severe nomophobia. In comparison, a study conducted in India among the general population it was found out that 98.8% were nomophobic and out of them 41.6% were mild, 42% were moderate and and the remaining 15.2% to be severely nomophobic (Kanmani et al 2017).

Out of the 100% nomophobic study population, females were found out to be severely affected, as 81.2% of the females were moderately-severe nomophobic as compared to a lesser proportion of 63.0% of males. Findings by Yildirim et al (2015) (58%) and Sharma et al (2015) (73%) indicates that females have higher proportion of nomophobia while Pavithra et al (2015) reports higher incidence of nomophobia in males (44.8%) compared to females (33.7%).

Frequent checking of the battery level, keeping the phone at close proximity when sleeping, using the phone during charging, concept of social life being non-existent without a smartphone and the realization that they spend more time on smartphone than they spend with family and friends had statistically significant associations with nomophobia while majority of the students being moderate-severe nomophobes.

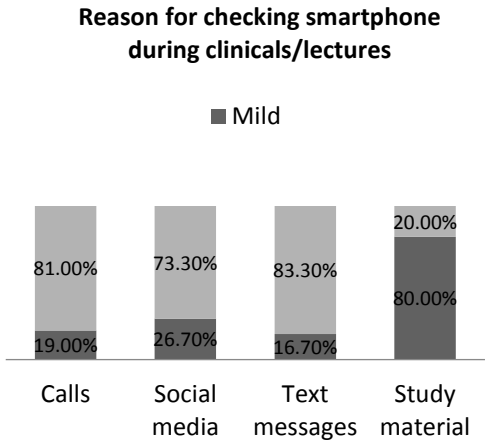
A larger proportion of moderate-severe nomophobes (38.6%) were having decrease in sleep quality and reduced total sleep time when the phone was used before retiring to bed compared to mild nomophobes (20%). This association between nomophobia and sleep quality was statistically significant. None of the mild nomophobes had woken up due to phantom vibrations during sleep to realize that the phone wasn't actually ringing/ vibrating while 2.9% of the moderate-severe nomophobes had experienced this issue which had adversely affected their sleep quality. These findings coincide with research done by Acharya et al (2013) among Indian undergraduates where 35.4% of the nomophobes had insomnia caused by smartphone use.

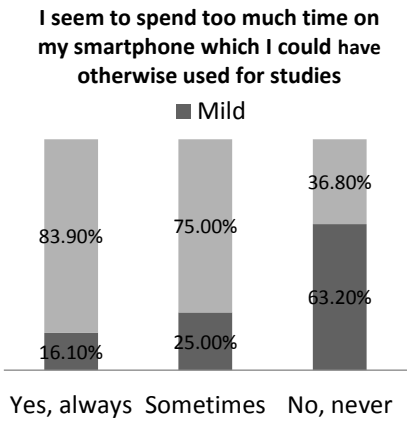
In a study conducted by Datta et al (2016) among medical students in India it was found out that 60.5% of the study population had false perception of ringing (ringxiety) while our study showed a much lesser percentage of 17.6%. Out of them 72% were moderate-severely nomophobic while the rest were mildly nomophobic.

In a study to assess the pattern of mobile phone usage and status of nomophobia amongst third year medical students in North India by Sharma et al (2015) it was found out that 83% of students experienced panic attacks when their mobile phone was misplaced. Headache and lethargy were the commonest side effects that were experienced by 61% of students when access to smartphones was restricted. In our study a considerable proportion of moderate-severe nomophobes (35.3%) had complained of irritability, restlessness and angry

emotions when unable to use the smartphone for any reason while only 20% of mild nomophobes complained of similar issues. This clearly indicates the widespread phenomenon of nomophobia could affect aspects of psychological wellbeing in a negative manner.

Diminished trans-active memory was high in both the groups of nomophobes with 70% of mild nomophobes and 72.3% of moderate-severe nomophobes complained of inability to remember important events, contacts and information without having access to the smartphone. In a research conducted by Kanmani (2015) in India 23% of the population had agreed that that they feel "weird" or a state where they "don't know what to do" without their mobile phone and 38 % of people felt annoyed if they could not look up to information on Smartphone or use it to its best capabilities when they want to.





Out of the 94.4% of the undergraduates who had stated that they usually check their smartphone during clinicals/ lectures 71.43% were moderate-severe nomophobic. Majority of the participants who used their phones during academics to look up study material were mild nomophobics (80.0%) while moderate-sever nomophobes were using for other activities. There was a strong association between reason for checking smartphone during clinicals/lectures and grade of nomophobia at 95% confidence level (p=0.028). Eighty-two-point five percent of the individuals who agreed to the statement that it will be more productive and useful if the academic staff allowed them to use smartphones during lectures/clinicals were revealed to be moderate to severe nomophobics (p=0.044). Majority (80.0%) of the individuals who felt less anxious by allowing to use smartphone during lectures/clinicals were also moderate to severe nomophobics.

It was interesting to note that students with higher degree of nomophobia have identified that their phone usage was excessive and tried to reduce it but have failed in doing so.

Addiction to smartphones and lack of adequate knowledge about the harmful effects could be the important reasons that have contributed to the increased incidence of some psychological health symptoms amongst the younger smartphone using undergraduates in Sri Lanka.

Conclusions

During this study the prevalence of nomophobia, the potential factors that may have led to the development of nomophobia and the effects of nomophobia on the psychological wellbeing of the study population were analysed. After the analysis it was concluded that the prevalence of nomophobia in the study population was 100.0% with female preponderance

People with higher degree of nomophobia were more frequent internet users, updated software as soon as available, checked smartphone during academics, never switched off their phones, kept their phones near the bed when sleeping, used it while charging, spent more time with the smartphone than with family and friends and was depended on it to maintain their social identity.

Higher degree of nomophobia was associated with reduced total sleep time and quality, waking up due to phantom vibrations, difficulty concentrating on studies, irritability, restlessness, less energetic, diminished trans-active memory, difficulty socializing directly with people, lonely and uneasy at public places without a smart phone.

Majority of the students have identified smartphones as a hindrance to studies and have attempted to reduce usage but have failed

Recommendations

Nomophobia is a novel field which had not yet been explored in Sri Lanka. The findings of this study stress the need for more researches to be done on the subject with a larger study population involving other communities.

There is a need to educate the society on this emergent concept as majority of the population despite being affected is unaware of this potential health hazard. People have realized the danger of the situation but at the same time have failed to make change to their behavior or have made unsuccessful attempts.

References

[1] Dasgupta, P., Bhattacherjee, S., Dasgupta, S., Roy, J.K., Mukherjee, A., Biswas, R. (2017) 'Nomophobic behaviors among smartphone using medical and engineering students in two colleges of West Bengal'.*Indian Journal of public Health* [online] 61 (3),1-7. Available from

http://www.ijph.in/article.asp?issn=0019-557X;year=2017;volume=61 ;issue =3;spage=199;epage=204;aulast=Dasgupta#ft3 [3rd December 2017]

[2] Kanmani,A., Bhavani,U., Maragatham,R. (2017) 'NOMOPHOBIA – An Insight into Its Psychological Aspects in India' *The International Journal of Indian Psychology* [online] 4 (2). Available from http://www.ijip.in/Archive/v4i2/18.01.041.20170402.pdf [3rd December 2017]

[3] Pavithra,M.B., Madhukumar,S., Murthy,M. (2015)'A study on nomophobia - mobile phone dependence, among students of a medical college in Bangalore' *National Journal of Community medicine*[online] 6 (2).Available from https://www.ejmanager.com/mnstemps/159/159-1455342507.pdf [3rd December 2017]

[4] Sharma,N., Sharma,P., Sharma.N., Wavre,R,.(2015)' Rising concern of nomophobia amongst Indian medical students'*. International Journal of Resrach in Medical Sciences* [online] 3 (3), 705-707.available from http://imsear.li.mahidol.ac.th/bitstream/123456789/165807/1/ijrms2015v3n3p705.pdf [3rd Decembr 2017]

[5] Yildirim,C., Correia, A.P.(2015) 'Exploring the dimensions of nomophobia: Development and validation of a self-reported questionnaire'. *Computers in Human Behaviour*[online] 49, 130-137. Available from <file:///C:/Users/user/Downloads/yildirim2015.pdf>[3rd December 2017]

KNOWLEDGE REGARDING PREVENTIVE PRACTICES, SIGNS AND EMERGENCY MEASURES IN FOREIGN BODY RELATED ENT INJURIES AMONG CAREGIVERS OF 1 TO 5-YEAR-OLD CHILDREN ATTENDING POLYCLINICS IN THE NUGEGODA MOH AREA FROM MARCH-JULY 2018

P.C. Yasawardene[1]*, W.H.M.Y. Withanage[1], H.B.D.N. Rajahewa[1], K.T. Wettasinghe[2]

[1]Faculty of Medicine, University of Colombo, Sri Lanka
[2] Department of Anatomy, Faculty of Medicine, University of Colombo, Sri Lanka
**Corresponding author (email: pcyasawardene@gmail.com)*

Introduction

The ear, nose and mouth provide entry points into the body into which external objects may be inserted or accidentally entered. A foreign body (FB) in the ear, nose, upper airway, mouth or throat is considered an ear, nose, throat (ENT) foreign body and they may cause problems ranging from slight irritation to life-threatening emergencies. Although ENT foreign body related injuries occur in adults, it is children who typically present with such problems and considered as preventable paediatric emergencies.

It is essential that caregivers understand the dangers posed by ENT FBs and practice adequate measures to keep them away from the reach of children. Caregivers must also be made aware of common clinical signs of inhalation of ENT foreign bodies enabling them to contact medical personnel early. Awareness of parents regarding ENT foreign bodies has been shown to be woefully inadequate in multiple countries [1-3]. Literature from the local setting with respect to the knowledge and practices among caregivers regarding ENT FB injuries and their prevention is profoundly limited.

This study was undertaken with the aim of assessing the state of knowledge regarding common ENT FBs, knowledge regarding the current practices undertaken to prevent ENT FB injuries and knowledge regarding the steps to be taken in case of ENT FB injuries (including the common signs, symptoms and emergency measures) among caregivers of 1 to 5-year-old children to help plan future public health education strategies and policy decisions regarding this important yet often neglected problem.

Materials and Methods

This cross-sectional analytical study was conducted among 120 caregivers of 1 to 5-year-old children attending the polyclinics of the Nugegoda Medical Officer of Health (MOH) area from March-July 2018. The sample size of 120 was selected

due to the time and resource constraints of conducting this study in the limited study period. Ethical clearance for the study was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Colombo. Formal permission was obtained for data collection from the MOH, Nugegoda. Caregivers having at least one child in the age range of 1 to 5 years in their household was included in the study provided the caregiver has lived in the household with the child for at least two thirds of a month, the child was not diagnosed with a physical disability and neither the caregiver nor the child was diagnosed as having a psychiatric illness.

Eligible participants were informed of the details of the research and written informed consent was obtained. Consecutive sampling was used and data collected using a structured, interviewer-administered questionnaire in Sinhala, Tamil and English as per the preference of the participant. Due to the lack of an existing validated tool for assessing parental knowledge of ENT FB injuries (at both local and international level), a new questionnaire was designed obtaining input from a consultant paediatric ENT surgeon. Questions were included on awareness of dangers posed by common aetiological agents of ENT FB injuries, source of knowledge on ENT FBs, current practices used to reduce risks of ENT FB injuries, knowledge of important signs of ENT foreign body injuries, socioeconomic and demographic factors.

An overall knowledge score was calculated incorporating all aspects of knowledge mentioned above. The median knowledge score was used as the cut-off to group the study population into “high knowledge” (≥median) and “low knowledge” (<median) groups for comparison. SPSS 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) was used for data analysis. Pearson’s Chi square test and independent samples t-test were used for analysing statistical significance of associations between overall level of knowledge regarding FB related ENT injuries with socioeconomic and demographic factors. A p-value < 0.05 was taken as statistically significant.

Results and Discussion

Total of 120 caregivers of children in the age range of 1 to 5 years participated in the study. Mean age of the study population was 35.5 years (standard deviation (SD)=9.7, 95%, confidence interval (CI)=33.8–37.2) with the minimum being 20 years and maximum being 74 years. Of the caregivers, 95 (79.2%) were females and majority (n=104, 86.7%) were the parents of the children. Therefore, the inferences drawn from the result of this study may be more valid for caregivers who are mothers. Prior studies have been conducted with study populations of entirely mothers [2] or parents of which 72% were females [3].

The most prominent single source of knowledge regarding harmfulness of FBs was the media (43.3%) followed by healthcare workers (25.0%) which is in contrast to the findings of Nichols, et al. [1] where the most common source of knowledge was physicians (67%) followed by books/ magazines (40%) and the Internet (25%). However, a study in the Dehiwala-Ratmalana MOH area has reported that knowledge on FB aspiration was "mainly" acquired from community midwives [4]. In the current study, participants were only allowed to choose the single most prominent source of their knowledge on foreign bodies (multiple responses were not allowed) while this was not so in the study by Nichols, et al. [1].

The knowledge score was approximately normally distributed with a mean of 34.48 (SD=7.94, CI=33.1-35.9). The minimum score obtained in this sample was one (obtained by only one person) while the maximum score obtained was 50 (obtained by only one person). The median score was 36. The distribution was found to be left skewed with a skewness of 0.997.

A large percentage (≥90%) of participants were aware of the danger of FBs (pen clips (95.8%), balloons (90.0%), seeds (99.2%) and popcorn (90.8%)). Coins and button batteries have been reported as the most commonly ingested foreign bodies in the local setting [5] and parental awareness on these were very high (99.2% and 97.5% respectively). This level of knowledge is higher compared to [1] specially for coins (97%), small batteries (93%), pen caps (92%), seeds (87%), balloons (84%) and popcorn (49%). In the present study, 73.3% and 75.8% of caregivers knew that pre-existing ear discomfort may increase insertion and habitual insertion may signal pre-existing ear disease respectively. No prior studies assessing the parental/caregiver's knowledge of this relationship could be found.

The majority (84.2%) had knowledge of age restrictions on toys and an even higher proportion (99.2%) knew about the presence of choking hazard warnings on toys. Amongst those who knew about age restrictions, though the majority (93.1%) checked recommended age ranges before giving toys to children under their care, more than one-third (35.1%) still admitted to letting the child have the toy even when the child fell outside the recommended age range. A limitation of the present study is that the reasons for exceeding age restrictions were not assessed. In households where more than one child was present, the younger sibling had unrestricted access to toys of the older sibling in 56.4% of cases. No prior studies assessing these behaviours could be found. The proportion agreeing with the statement that children should not be allowed to walk/laugh while eating was 64.2% in the present study and was low when compared to 81.9% [2]

and 76% [3] in prior studies. This may be a potential target for future health education efforts.

In the present study, majority of caregivers correctly identified choking/coughing (77.5%) and stridor/wheezing (70.8%) as signs indicative of foreign bodies in the throat and/or airway. In a prior study, sudden choking and sudden cough have been identified as possible signs by 74% and 68% respectively [3] while another study has reported 27.7% and 41.8% of mothers have lacked this knowledge regarding choking and cough respectively [2]. Painful swallowing was correctly identified by 80.8% of caregivers while chronic ear pain and ear discharge were correctly identified by 85.0% and 77.5% of caregivers respectively. However, only 42.5% of caregivers correctly identified unilateral nasal discharge as a sign indicative of foreign bodies in the nose. There have been no documented prior studies assessing the knowledge of caregivers on these aspects.

Previous local study has reported an "average" overall knowledge of parents regarding foreign body aspiration (58.9%), while the majority (59.9%) have had a good knowledge on first aid management [4]. In the present study, on a scale of low (=1) to high (=5) agreement, on average, there was good agreement (4.31) with not attempting any form removal by oneself and even higher agreement (4.35) with immediately seeking medical attention. There was a relatively low agreement (1.64) with disregarding a possible injury if the child appears normal. Not attempting removal without medical help is important as improper attempts may push the foreign body further in. A previous study in Saudi Arabia has reported that 98% of parents agreed with seeking immediate medical treatment and 20% thought absence of signs is reassuring [3].

A statistically significant difference was seen with the mean knowledge score being lower among caregivers who did not manage to complete their study up to the level of the G.C.E. ordinary level examination as compared to those who achieved a level of education equal to or above the standard of the G.C.E. ordinary level examination ($t=-2.191$; $df=118$; $p < 0.05$). However, a previous study conducted in the Dehiwala-Ratmalana MOH area has found no association between the education level of parents with the awareness of risk, recognition, prevention, first aid management or overall knowledge of FB aspiration [4]. Nichols, et al. [1] (United States) and Alqudehy and Alsheef [3] (Saudi Arabia) have also failed to demonstrate a statistically significant association between parental education and knowledge regarding FB aspiration. The difference in findings may be due to the wider range of FB ENT injuries considered in the current study (as opposed to only FB aspiration) and to the lack of validated questionnaires.

No statistically significant association between knowledge of a caregiver on foreign body related ENT injuries with the age, gender, relationship to the child under their care, the monthly income or the total monthly income of the entire family was seen. Previous studies on parental knowledge on aspiration have also failed to show an association between age [2, 3] and household income [1]. Presence of prior foreign body injuries in at least one child in the family was also not associated with an improvement in overall knowledge of caregivers. A positive association between prior instruction and increased knowledge on foreign body aspirations have been demonstrated by [1].

Conclusions and Recommendations

In conclusion, the level of knowledge of the caregivers of children of one to five years of age in the local setting regarding foreign body related ENT injuries was at a similar or higher level when compared to the previous studies carried out in the local and international context. The level of knowledge of the caregivers regarding preventive measures and emergency measures regarding foreign body related ENT injuries in the local setting is comparable to that reported in other regions. The level of education of the caregivers correlates with the level of knowledge regarding foreign body related ENT injuries. Media is the most common source of knowledge regarding foreign body related ENT injuries.

Due to its prominence as a source of knowledge regarding ENT foreign body injuries, media may be used as an effective way of increasing the awareness among caregivers regarding prevention of such injuries. The parents and caregivers should be educated regarding hazards posed by toys, importance of limiting toys to the child's recommended age range and proper storage of toys of elder siblings without mixing with those of younger siblings. Health education of caregivers should emphasise on proper feeding practices, including the avoidance of force feeding and not feeding the child when he/she is walking or laughing.

Acknowledgements

This research was carried out as a requirement of the Community Stream undergraduate academic programme of the Faculty of Medicine, University of Colombo, Sri Lanka.

References

[1] B.G. Nichols *et al.*, "Pediatric exposure to choking hazards is associated with parental knowledge of choking hazards," *International Journal of Pediatric Otorhinolaryngology,* vol. 76, no. 2, pp. 169-173, 2012.

[2] O. Higuchi, Y. Adachi, Y.S. Adachi, H. Taneichi, T. Ichimaru, and K. Kawasaki, "Mothers' knowledge about foreign body aspiration in young children,"

International Journal of Pediatric Otorhinolaryngology, vol. 77, no. 1, pp. 41-44, 2013.

[3] Z. Alqudehy and H. Alsheef, "Parental Knowledge of Foreign Body Aspiration: A Comparative Study between Saudis and Other Nations," *Journal of Otolaryngology-ENT Research,* vol. 1, no. 2, 2015.

[4] S.M.D.R. Samarakoon, P.A.M.T. Polpitiya, S.P.P.I.M.N.D. Paramanayaka, T.K. H.P. Apasara, H.M.P. Herath, and G. Jayatilaka, "Parental awareness of risk factors, recognition and first aid management of accidental foreign body aspiration in children aged 1-5 years in Dehiwala-Ratmalana area, Colombo, Sri Lanka," in *9th Asia Pacific Global Summit on Healthcare & Immunology*, Kuala Lumpur, Malaysia, 2017, p. 47.

[5] B. Branavan, R. Dias, T. Kumari, and M. Samarasinghe, "Foreign body ingestion in children: Single institutional experience in Sri Lanka," *Sri Lanka Journal of Child Health,* vol. 43, no. 2, 2014.

A PRELIMINARY INVESTIGATION OF THE ANTIBACTERIAL ACTIVITY OF *Punica granatum* IN SRI LANKA

S. Dias* and M. A. Dharmaratne

School of Science, BMS, 591, Galle Road, Colombo 06, Sri Lanka
**Corresponding author (email: sajani@bms.edu.lk)*

Introduction

The growing interest and demand for natural preservatives has evoked interest in determining the antimicrobial and antioxidant activity of herbal extracts (Bin *et al*., 2011). Of particular interest, is the commercially grown pomegranate (*Punica granatum*) which comes from the Punicacae family. As compared to imported pomegranate cultivars, the Sri Lankan cultivars appear much smaller in size. The arils of the fruit are far smaller and have an acidic taste, as opposed to the usual sweetness found in pomegranates. Due to these reasons, the local varieties are less consumed.

Pomegranates have been known to demonstrate antimicrobial effects against various pathogenic species, due to their phenolic constituents. The rich source of tannins, flavonoids, phenolics and proanthocyanidins make up the bioactive compounds of the fruit and are responsible for the therapeutic effects that are elicited (Howell and D'Souza, 2013).

The general objective of this preliminary study was to determine the antibacterial activity of *Punica granatum* in Sri Lanka. The specific objectives of this study were:

(a) To determine the antibacterial activity of the peel and juice of the pomegranate using different concentrations by the disk diffusion method, against *Staphylococcus aureus* and *Escherichia coli*.

(b) To determine the minimum inhibitory concentration in this study against the same bacterial strains.

Materials and Methods

Chemicals and reagents

Methanol (99.9%), distilled water, 70% ethanol, barium chloride, sulphuric acid, nutrient broth powder, Mueller Hinton agar powder, Nutrient agar powder, Gentamycin impregnated disks (Sigma-Aldrich), potassium dihydrogen phosphate, disodium phosphate, sodium chloride, potassium chloride, phosphate buffered saline and saline water. The Control of Substances Hazardous to Health (COSHH) and bioCOSHH forms were filled out to acquire approval for the bacterial strains and chemical reagents.

Sample collection and preparation of extracts

The pomegranates were obtained from a local garden and were immediately washed and peeled. The juice was obtained from the arils of the fruit. The peels were air dried and blended into a fine powder, while the juice was stored at 4°C until further use. Both the extracts were then subjected to a methanolic extraction.

The bacterial strains *Staphylococcus aureus (ATCC -25923)* and *Escherichia coli (ATCC- 35218)* were sub cultured on nutrient agar and were left to incubate at 37°C for 48 hours. During the disk diffusion assay, bacteria from the sub culture were dissolved in saline solution under aspetic conditions. This was compared against the 0.5 MacFarland standard to obtain a bacterial concentration between 1-2 x 10^8 colony forming units per milliliter (CFU/ml).

The disk diffusion assay was carried out to determine the antimicrobial activity of the pomegranate peel and juice extracts against *Staphylococcus aureus* and *Escherichia coli*. Under sterile conditions, 6mm filter paper disks were dipped into three different concentrations (10mg/ml, 100mg/ml and 150mg/ml) for each extract and were carefully placed onto the surface of inoculated agar plates. The positive control used in this assay was a Gentamycin disk, while the negative control was phosphate buffered saline (PBS). After the period of incubation, the diameters of the zones of inhibition were measured in millimeters using a ruler. The assay was performed in triplicates for each extract, the pomegranate peel and juice (Ramadan *et al*., 2015). The zones of inhibition were expressed as mean±SD.

The MIC for both extracts followed the broth dilution method (Ramadan *et al*., 2015). Solutions from the 10mg/ml concentration of the peel and juice extracts were serially diluted (two fold) to make exact concentrations of 0.62, 1.25, 2.50 and 5.00mg/ml. Each tube contained the bacterial isolate (adjusted to 0.5 MacFarland standard), plant extract and nutrient broth. The positive control included the nutrient broth with the bacterial isolate and the negative control included phosphate buffered saline. The tubes were incubated at 37°C for 24 hours. The MIC was recorded as the lowest concentration of the pomegranate extract to inhibit the growth of bacteria and show no turbidity after 24 hours of incubation at 37°C. Further, 300µl from each test tube was dispensed into a 96 well microplate. The plate was then placed into the EZ READ 400 microplate reader and the O.D of cultures were recorded at 620nm.

Statistical analysis

All available data were entered into a database using the SPSS statistical software (SPSS for windows, version 21.0, IBM Corporation, NY, USA) and were analyzed using one-way analysis of variance (ANOVA) to compare the mean inhibitory zones. The values were considered to be statistically different at $p<0.05$.

Results and Discussion

The antibacterial activity of the methanolic pomegranate peel and juice extracts were investigated against *Staphylococcus aureus* and *Escherichia coli.* The results presented in table 1 and 2 indicated that both extracts displayed antibacterial activity against both bacterial strains at concentrations of 10mg/ml, 100mg/ml and 150mg/ml. The extracts displayed zones of inhibition in a dose-dependent manner, with an exception for the juice extract tested against *Escherichia coli*.

Table 1. Zones of inhibition for pomegranate peel and juice extracts against *Staphylococcus aureus*

Bacteria		
Staphylococcus aureus		
	Mean Inhibitory zones (mm)	
Concentration (mg/ml)	**Pomegranate Peel extract**	**Pomegranate Juice extract**
10mg/ml	9.66±2.51mm	8.33±0.57mm
100mg/ml	12.33±3.78mm	12.00±1.00mm
150mg/ml	17.00±0.00mm	14.66±1.52mm

Data expressed as mean±SD

Table 2. Zones of inhibition for pomegranate peel and juice extracts against Escherichia coli

Bacteria		
Escherichia coli		
	Mean Inhibitory zones (mm)	
Concentration (mg/ml)	**Pomegranate Peel extract**	**Pomegranate Juice extract**
10mg/ml	10.66±1.15mm	8.33±0.57mm
100mg/ml	13.00±1.00mm	17.33±4.61mm
150mg/ml	17.33±0.57mm	13.66±1.52mm

Data expressed as mean±SD

The data in table 2 indicates the zones of inhibition recorded against *Escherichia coli* are higher than for S*taphylococcus aureus*, with respect to the juice extract. Table 1 and 2 also indicates that the peel extract had a higher zone of inhibition against *Escherichia coli* than *Staphylococcus aureus*. The overall results from the disk diffusion assay conclude that the Sri Lankan cultivars exert a greater antibacterial activity towards gram- negative bacteria such as *Escherichia coli*.

Results from the minimum inhibitory concentration assay in table 3 indicated that the minimum inhibitory concentration recorded for both peel and juice extracts were observed to be 5mg/ml for *Staphylococcus aureus*. In comparison, in table 4, the MIC for the peel extract against *Escherichia coli* was recorded as

1.25mg/ml, whereas the juice extract demonstrated the range to be in 10-5mg/ml. The results from table 4 indicate that a low concentration of 1.25mg/ml is sufficient enough to inhibit *E.coli*, rather than the juice extract which can inhibit at 5mg/ml. This finding is unique to Sri Lankan cultivars as gram-negative bacteria is effectively inhibited than gram-positive bacteria.

Table 3. Minimum inhibitory concentration of pomegranate extracts for *Staphylococcus aureus*.

MIC for *Staphylococcus aureus* at 620nm					
	Concentration (mg/ml)				
	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml
Pomegranate Peel extract	**-**	**-**	**+**	**+**	**+**
Pomegranate Juice extract	**-**	**-**	**+**	**+**	**+**

Note: - indicates = no turbidity, + indicates = turbidity

Table 4. Minimum inhibitory concentration of pomegranate extracts for *Escherichia coli*

MIC for *Escherichia coli* at 620nm					
	Concentration (mg/ml)				
	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml
Pomegranate Peel extract	**+**	**+**	**+**	**-**	**+**
Pomegranate Juice extract	**-**	**-**	**+**	**+**	**+**

Note: - indicates = no turbidity, + indicates = turbidity

With respect to the structure of Gram-negative and Gram-positive bacteria, the results from this present investigation are contradictory. Gram-negative bacteria displays resistance towards antibacterial agents due to the presence of an outer membrane, which is not present in Gram-positive bacteria and therefore many studies have reported an increased susceptibility of Gram-positive bacteria than Gram-negative bacteria to antibacterial agents (Mahboubi *et al*., 2015). Since the present study demonstrated the ability of the peel extracts to inhibit Gram-negative bacteria (*E.coli*) at the highest concentration of 150mg/ml, this could very likely be due to the presence of an increase in the polyphenol constituents in Sri Lankan cultivars of pomegranates as opposed to imported cultivars.

This is a promising opening to conduct more antibacterial tests for Sri Lankan cultivars, because it would be especially helpful in combating harmful *E.coli* strains that cause human gut diseases, such as Shiga toxin producing *E.coli.* Pomegranate extracts are known to contain metabolic toxins or broad spectrum

compounds that give rise to their antibacterial activity; furthermore, this study utilized methanolic extractions of pomegranate peel and juice. It has been reported that the use of hydrophilic extracts act as better solvents for the polyphenolic constituents present in the extracts (Devatkal *et al*., 2013).

Conclusions and Recommendations

Throughout the present study, *Staphylococcus aureus* and *Escherichia coli* demonstrated increased susceptibility to both extracts in general. The pomegranate peel extracts demonstrated a higher antibacterial activity in the disk diffusion assay and MIC assay in comparison to the pomegranate juice extract. The MIC assay further affirmed that the peel extract was able to inhibit *Escherichia coli* far better at a lower MIC (1.25mg/ml) than the juice extract MIC (5mg/ml). This study demonstrates that Sri Lankan cultivars can inhibit gram-negative bacteria more effectively than gram-positive bacteria. An integrated approach would be required to utilize the pomegranate fruit and its components in the development of its use as a natural medicinal product.

References

[1] Bin, S., Cai, Y.Z., Brooks, J.D. & Corke, H. “Potential application of spice and herb extracts as natural preservatives in cheese.” *Journal of Medicinal Food*, vol.14, pp.284-290, 2011.

[2] Howell, A. B. & D’Souza, D.H. “The pomegranate: Effects on bacteria and viruses that influence human health.” *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, pp. 1-11, 2013.

[3] Ramadan, H., Min, B., Tiwari, A.K., Reddy, G., Adesiyun, A., Hinton Jr., A. & Abdela, W. “Antibacterial activity of pomegranate, orange and lemon peel extracts against food-borne pathogens and spoilage bacteria *In vitro* and on poultry skin.” *International Journal of Poultry Science*, vol. 14, pp. 229-239, 2015.

[4] Mahboubi, A., Asgarpanah, J., Sadaghiyani, P.N. & Faizi, M. “Total phenolics and flavonoid content and antibacterial activity of *Punica granatum* L. var. *pleniflora* flowers (Golnar) against bacterial strains causing foodborne diseases.” *BMC Complementary and Alternative Medicine*, vol. 15, pp. 1-7, 2015.

[5] Devatkal, S.K., Jaiswal, P., Jha, S.N., Bharadwaj, R. & Viswas, K.N. “Antibacterial activity of aqueous extract of pomegranate peel against *Pseudomonas stutzeri* isolated from poultry meat.” *Journal of Food Science and Technology*, vol. 50, pp. 555-560, 2013.

PRODUCTION OF AN ENTOMOPATHOGENIC NEMATODE, *Acrobeloides longiuterus* USING ARTIFICIAL SOLID MEDIA

N. Thiruchchelvan*, G. Thirukkumaran, G. Mikunthan

Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Sri Lanka
**Corresponding author (email: thiruchchelvann@univ.jfn.ac.lk)*

Introduction

Acrobeloides longiuterus (Nematoda: Cephalobidae) is a free-living nematode [not a true member in the entomopathogenic genera (*Steinernema* and *Heterorohabditis*)] isolated from soils of Northern Sri Lanka using an insect-baiting technique. The propensity of this nematode to kill insect pests has been tested with many agriculturally important pests under *in-vitro* and field conditions [1], [2] and it shows the potential to be used as a biological control agent in future. However a bio-control agent is required to be used in large scale production and readily available in required quantity with low cost of production throughout the year for the entrepreneurs and farmers [3]. Therefore, a study was carried out with an objective; to evaluate the production efficiency of *A. longiuterus* on different solid media and its quality on insect-pathogenicity.

Materials and Methods

In-vitro culturing of Acrobeloides longiuterus

Culturing of *A. longiuterus* in solid media was tested using modified Wout's medium [4] and newly developed a medium. All the ingredients shown in the Table 1 were added into the conical flask and contents were mixed with 1 L of distilled water using a magnetic starrier at a temperature of 75 ^{o}C for five minutes. Conical flasks plugged with cotton were sterilized using an autoclave at 121 ^{o}C, 1.054 kg/cm^2 for 20 minutes.

The culture medium was transferred into a 90 mm diameter petri-dish (15mL/petri-dish) under aseptic conditions. Subsequently, symbiotic bacteria isolated from nematode was inoculated into each medium and incubated for four days. On the grownup bacterial culture, a 6 mm hole was made in the middle using a cork borer. Subsequently 100 Infective Juveniles (IJs) were introduced into the hole. This was replicated four times. Continued observation and monitoring were conducted until the nematode development was visible. IJs were collected using White trap techniques following 15 days inoculation.

Pathogenicity of Acrobeloides longiuterus against Tribolium castaneum

A moisture chamber assay was used to test the quality of *A. longiuterus* using *in-vitro* production methods as described by Kaya and Stock [5]. *T. castaneum* larvae and pupae were exposed to the different concentrations. An experiment was arranged with four different concentrations of 50, 100, 150 and 200 IJs/mL/petri-

dish and distilled water was used as the control. Moisture chambers containing 10 larvae and 10 pupae were separately used to apply the above concentrations and, were replicated four times. Pupal and larval mortality were recorded on 2nd and3rd days after inoculation, respectively.

Statistical analysis

Data were analysed using one-way ANOVA and mean separation was carried out according to the Fisher LSD method at the 95 % of the confidence interval. Probit analysis was used to calculate the LC_{50} and LC_{90} values using the software of Minitab 17.

Results and Discussion

Acrobeloides longiuterus production on different media under in-vitro conditions

In-vitro production of *A. longiuterus* using modified Wout's medium and other media composition are given in the Table 1. Highest IJs were produced in the medium IV (Nutrient agar, Soy flour, sun oil and glycerol), which was found to be 2.01 X 10^6 IJs/15mL, followed by 8.98 X 10^5, 5.5X 10^5 and 3.43 X 10^5 IJs/15mL respectively. This yield of IJs comparably high with the results obtained by the El-Sadawy [4]. He reported that, *Steinernema carpocapsae, S. scapterisci*, *S. riobrave, S. carpocapsae, S. abbasi, S. glaseri* and *S.* spp, 5, 8, 8.5, 5.5, 6, 4 and 3 million of IJs yielded/kg of modified Wout's medium, respectively. However the compositions of the media and cultured nematodes species are different.

Quality testing of Acrobeloides longiuterus against Tribolium castaneum

IJs production in different *in-vitro* media was tested against *T. castaneum*. Mortality of the larval and pupal stages at different concentrations is given in the Table 2. Mortality of larvae at all concentrations of *A. longiuterus* IJs/mL/petri-dish was significantly different from the untreated control. Highest mortality of larvae and pupae (92.5%) was recorded at the concentration of 200 IJs/mL/petri-dish. LC_{50} and LC_{90} of the larvae were calculated as 48.55 and 210.42 IJs/mL/petri-dish, respectively.

All the concentrations of *A. longiuterus* IJs against pupal mortality was significantly different from the control. Pupal mortality rates of 65, 75 and 90 % were recorded at the concentrations of 50, 100, 150 IJs/mL/petri-dish, respectively. LC_{50} and LC_{90} of the pupae were calculated as 32. 94 and 173.97 IJs/mL/petri-dish, respectively. Pupal mortality was caused by the IJs produced from the *T. castaneum* larvae and yielded a LC_{50} of 10.64 IJs/mL/petri-dish.

Table 1. Production of infective juveniles' of *Acrobeloides longiuterus* under *in-vitro* conditions using different media

Components	Media			
	I	II	III	IV
Nutrient Broth (g)	16	16	-	-
Nutrient agar (g)	-	-	32	32
Bacteriological Agar (g)	12	12	-	-
Yeast extract (g)	6	6	-	-
Soy flour (g)	7	7	7	7
Sun flower oil (mL)	5	5	5	5
Glycerol (mL)	5	5	5	5
NaCl (g)	0.5	-	0.5	-
$CaCl_2$ (g)	0.21	-	0.21	-
KH_2PO_4 (g)	2	-	2	-
Distilled water (L)	1	1	1	1
IJs/15 mL of medium* (10^6)	0.55 ± 0.12^{bc}	0.34 ± 0.05^{c}	0.90 ± 0.27^{b}	2.01 ± 0.43^{a}
IJs/mL of medium (10^3)	36.67	22.88	59.87	134.21

** Each value represents the mean value from four replicates. . *Values having the same letter are not significantly different according to the Fisher LSD at 95 % confidence interval*

Table 2. Mean mortality of *Tribolium castaneum* larvae and pupae by *Acrobeloides longiuterus* produced from *in- vitro* media

Concentration IJs/mL/petri-dish	Mean Mortality*	
	Larva	Pupa
0	$^{d}0.25\pm0.500$	$^{d}0.5\pm0.577$
50	$^{c}5.25\pm0.500$	$^{c}6.5\pm0.577$
100	$^{b}7.25\pm0.500$	$^{b}7.5\pm0.577$
150	$^{b}8.00\pm0.816$	$^{a}9.00\pm000$
200	$^{a}9.25\pm0.500$	$^{a}9.25\pm0.5$
LC_{50}	48.55	32.94
LC_{90}	210.42	173.97

** Each value represents the mean value from four replicates. Values having the same letter in a column were not significantly different according to the Fisher LSD at 95 % confidence interval*

Conclusions and recommendations

Acrobeloides longiuterus successfully cultured in a developed nutrient agar based medium (2 million IJs/15 mL) and without losing their insect-killing (more than 92 %) quality. Therefore, the nutrient agar based medium is better for this nematode multiplication in a small scale level and it can be used for the pest management

programs. However, the testing in different formulations in fields need to be conducted before the commercialization.

References

[1] N. Thiruchchelvan, G. Thirukkumaran and G. Mikunthan. "Bio-efficacy of entomopathogenic nematode, *Acrobeloides longiuterus* against spotted beetle (*Epilachna vigintioctopunctata*)."*Proceeding of the SLCARP international agricultural research symposium*, 2018, pp.46.

[2] N. Thiruchchelvan, G. Thirukkumaran and G. Mikunthan. "Distribution of entomopathogens in northern Sri Lanka and their potential as bio-agents", *Proceeding of 7thyoung scientist forum symposium, National Science and Technology Commission*, vol. 7, no. 1, 2018, pp. 162-166.

[3] S.S. Hussaini, J.S. Kavitha, and M.A Hussain. "Mass production of a native *Steinernema* sp. (SSL2) PDBC EN 13.21 (Nematode: Steinernematidae) on different artificial media." Indian Journal of Plant Protection, vol. 28, no. 1, pp. 94-96, 2000

[4] H.A. El-Sadawy, "Mass production of *Steinernema* spp. on in-vitro developed solid medium," World Applied Sciences Journal, vol. 14, no. 6. pp. 803–813, 2011.

[5] H.K. Kaya and S. Patricia Stock, "Techniques in insect nematology," in Manual of Techniques in Insect Pathology, 1997, pp. 281–324.

ASSOCIATION OF *TP53* CODON 72 POLYMORPHISM WITH SELECTED HUMAN CANCER TYPES IN SRI LANKA

V. Manoharan[1], E.H. Karunanayake[1], K.H. Tennekoon[1], S. De Silva*[1], K. De Silva[2], P. Angunawela[3], J. Lunec[4]

[1]*Institute of Biochemistry Molecular Biology and Biotechnology, University of Colombo, Sri Lanka*
[2]*National Cancer Institute, Maharagama, Sri Lanka*
[3]*Department of Pathology, Faculty of Medicine, University of Colombo, Sri Lanka*
[4]*Northern Institute for Cancer Research, Newcastle University, United Kingdom*
**Corresponding author (email: sum@ibmbb.cmb.ac.lk)*

Introduction

TP53 is 20 kb long tumour suppressor gene containing 11 exons and 10 introns and located on the short arm of human chromosome 17. p53 is a phosphoprotein made up of 393 amino acid (55 kDa) expressed by *TP53* gene [1]. It functions as the ”guardian of the genome”, involved in the regulation of the growth of genetically altered cells by controlling cell cycle arrest, apoptosis, senescence and also facilitates repair of the altered genes [2].

Codon 72 variant (p.Arg72Pro) is a well-studied Single Nucleotide Polymorphism in exon 4 of *TP53*, resulting in the expression of either Arginine - R (CGC) or Proline - P (CCC) residues. Activities of the resultant two proteins with these residues are different, which affect the function of the gene. This polymorphism creates three genotypes: Homozygous Arginine (R/R), Heterozygous (R/P), and Homozygous Proline (P/P). Several studies have been conducted to evaluate the effect of these three genotypes in the occurrence of different cancers worldwide. p53 protein that contains Arginine residue has been identified to have more preventive effect, as it has a greater pro-apoptotic effect, while protein containing Proline residue is associated with increased risk of various cancers [3].

Significant ethnic differences have been observed in allele frequencies of codon 72 polymorphism and this ethnic difference might have a pronounced effect on cancer risk between different populations [3]. This is a preliminary study focused on the association of codon 72 polymorphism with different types of cancer in Sri Lanka. We have selected head and neck cancers and breast cancer in this study, as those are more prevalent cancer types in Sri Lankan males and females respectively. In addition, we have also selected colorectal cancer as it is a cancer type reported with a higher frequency of *TP53* somatic mutations.

Materials and Methods

Patient recruitment and sample processing

Ethical approval was obtained from the Ethics Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka (EC/14/160). Patients with head and neck cancer (N=44), breast cancer (N=26) and colorectal cancer (N=17) who had undergone surgical resection at the National Cancer Institute, Sri Lanka from 2015 – 2018 were recruited for this study. Written informed consent from the study participants was obtained prior to recruitment. Socio – demographic and clinical data were obtained from study participants using questionnaires and by reviewing their medical reports. A majority of our patient population represents the Sinhalese ethnicity. Healthy controls (N=20; 10 males, 10 females) with no personal/ family history of any cancer were also recruited for this study.

Surgically excised tumour tissues were collected and immediately placed in Allprotect® Tissue Reagent (QIAGEN, cat no. 76405, Hilden, Germany) and stored at -20^{0}C until processed.

Genomic DNA was extracted from the excised tumour tissue of patients and from peripheral venous blood of healthy controls. Disruption of tissue specimens was done in liquid nitrogen using mortar and pestle followed by homogenization using QIAshredder (QIAGEN, cat. no. 79654). Tissue DNA was extracted from homogenized sample using All prep DNA/ RNA/ Protein mini kit® (QIAGEN, cat. no. 80004) following manufacturer's protocol and stored at – 20^{0}C until used. Genomic DNA was extracted from blood using the modified protocol described by Miller et al, 1998 [4].

Polymerase Chain Reaction and Direct Sequencing

A primer pair (Forward – 5'CCTGGTCCTCTGACTGCTCT3', Reverse - 5'GCCAGGCATTGAAGTCTCAT3') covering exon 4 was designed using the NCBI/ Primer-BLAST tool (https:// www.ncbi.nlm.nih.gov/tools/primerblast/ index.cgi? ORGANISM=9606&INPUT_SEQUENCE=NM_001618.3). Polymerase Chain Reaction (PCR) was performed using a final volume of 25µl containing 100ng genomic DNA, 3.5mM $MgCl_2$, 1X Green GoTaq® reaction buffer [10mM Tris-HCl (pH 8.3) and 50mM KCl], 2.5mM dNTPs (Promega Corporation, Madison, WI, USA), 5pmols of each primer (IDT Integrated DNA Technologies, Coralville, Iowa, USA) and 1 unit of GoTaq® Flexi DNA polymerase (Promega). PCR reactions: 94 °C for 7 min, 33 cycles of 94 °C for 1 min, 64°C for 1 min and 72 °C for 1 min and final extension at 72 °C for 10 min were performed using a thermo cycler (Veriti Thermal Cycler, Thermo Fisher Scientific). The annealing temperature and $MgCl_2$ concentration were optimised for the primer set.

PCR products were purified using the Wizard® SV Gel and PCR Clean-Up (Promega) and purified products were directly sequenced using BigDye® Terminator v3.1 kit (Thermo Fisher Scientific, Waltham, MA USA) and Applied Biosystems™ 3500Dx Genetic Analyzer (Thermo Fisher Scientific). Sequence variants detected were reconfirmed by performing a second PCR and direct sequencing.

Sequencing results were analysed to identify codon 72 polymorphisms using a human *TP53* NCBI database reference sequence (Genbank accession number - NC_000017), via Bio Edit® software and further confirmed by Mutation Surveyor®V4.0.9 and Alamut® Visual 2.7.2 Documentation.

Statistical analysis

Data were analysed using a chi-squared (X^2) test to assess the significance of the association of three genotypes between cases and controls. A p-value < 0.05 was considered statistically significant. The association between the *TP53* codon 72 polymorphism and cancer risk was estimated by computing the Odds ratio (OR) and 95% confidence interval (CI) was used to estimate the precision of the OR. Hardy – Weinberg equilibrium was also applied using the chi-squared (X^2) test to calculate whether the genetic variation of a population was at equilibrium or not.

Results and Discussion

Genetic data obtained are summarized in Table 1. In the present study, R/R, R/P and P/P genotype distribution among head and neck cancer patients was 10 (22.7%), 15 (34.1%) and 19 (43.2%), breast cancer patients was 10 (38.5%), 10 (38.5%) and 6 (23%), and colorectal cancer patients was 5 (27.8%), 9 (50%) and 4 (22.2%) respectively. In healthy controls, the percentage of R/R, R/P and P/P was 7 (35%), 6 (30%) and 7 (35%) respectively. There was no significant difference in the prevalence of different genotypes between any type of patients and healthy controls.

Allele Frequencies of R and P were 0.4/0.6, 0.42/0.58, 0.47/0.53 in the head and neck, breast and colorectal patient cohorts respectively, while the two alleles were equally distributed among the healthy controls. Alleles were in Hardy-Weinberg equilibrium within each group and did not significantly differ between cancer patients and healthy controls.

The implication of this polymorphism in cancer risk and prognosis is controversial, with some earlier studies reporting that p53 protein containing the codon 72 Arginine form has a greater apoptotic potential, while others have failed to replicate these findings.

However, in the present study, in head and neck cancer, the Proline allele was found to be more prevalent in patients than in controls and carriers of the

Proline alleles were positively associated with an increased risk of head and neck cancer. This effect was observed for both heterozygote individuals (R/P) with an OR of 1.75 and for homozygotes carriers (P/P), with an OR of 1.9, but p values were not significant in both cases. Nevertheless, breast and colorectal cancer showed no positive association of cancer risk with Proline alleles.

Table1. Frequency distribution of *TP53* codon 72 polymorphism between cases and controls and its association of with risk of cancer

Genotype/ Allele	Cases	Control	p-value	OR (95% CI)
Head and neck				
Genotype				
Arg/Arg	10	7	1	1 (Reference)
Arg/ Pro	15	6	0.417	1.750 (0.453 – 6.768)
Pro/Pro	19	7	0.332	1.900 (0.519 – 6.955)
Allele				
Arg	35	20	1	1 (Reference)
Pro	53	20	0.280	1.514 (0.713 – 3.214)
Breast				
Genotype				
Arg/Arg	10	7	1	1 (Reference)
Arg/ Pro	10	6	0.829	1.167 (0.288 – 4.727)
Pro/Pro	6	7	0.492	0.600 (0.140 – 2.575)
Allele				
Arg	30	20	1	1 (Reference)
Pro	22	20	0.463	0.733 (0.320 – .680)
Colorectal				
Genotype				
Arg/Arg	5	7	1	1 (Reference)
Arg/ Pro	9	6	0.346	2.100 (0.448 – 9.836)
Pro/Pro	4	7	0.795	0.800 (0.149 – 4.298)
Allele				
Arg	19	20	1	1 (Reference)
Pro	17	20	0.809	0.895 (0.363 - 2.204)

OR – Odd Ratio, CI – Confidence Interval

Conclusion and recommendation

Though odd ratio values showed positive association of Proline allele with the increased risk of head and neck cancer, the difference is not statistically significant. This may be due to small number of samples. Thus further studies with a larger sample size are needed to evaluate the potential effect of the *TP53* codon 72 polymorphism in the risk of cancer.

References

[1] DP Lane. p53, Guardian of the genome. Nature, 358, 15- 16, 1992.
[2] LR Livingstone, AWhite, J Sprouse, E Livanos, T Jacks, TD Tlsty. Altered cell cycle arrest and gene amplificationpotential accompany loss of wild-type p53. Cell, 70, 923 -935, 1992.
[3] L Bouaoun, D Sonkin, M Ardin, M Hollstein, G Byrnes, J Zavadil, M Olivier. TP53 Variations in Human Cancers: New Lessons from the IARC TP53 Database and Genomics Data. Human Mutation,37(9), 865-76, 2016.
[4] S Miller, D Dykes, H Polesky. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Reserch, 16, 1215, 1988.

Acknowledgment

Financial assistance by National Research Council under the grant number 15-033

Author Index

Panel of Reviewers

Prof. H.M.A.M.C. Herath
Prof. L.V. Athiththan
Prof. T. Sivanantheawerl
Prof. S. Samita
Prof. L.D.B. Suriyagoda
Prof. C. Dissanayake
Prof. R. Perera
Dr. Thushari Dissanayake
Dr. Chnithika Gunasekara
Dr. Jananie Kottahachchi
Dr. Sujatha Pathirage
Dr. T.C. Bamunuarachchige
Dr. P.H.P. Presanna
Dr. N. M. Adasooriya
Dr. P. Ranasinghe
Dr. S. D.N.K. Bathige
Dr. U.Hettiaratchi
Dr. T. Bandara
Dr. B.P. Galhena
Dr. N.D. Withanage
Dr. S. Mahavithanage
Dr. P. Athiththan
Dr. Dinum Perera
Dr. Chandrika Perera
Dr. Dharshani Bandupriya
Dr. W.S.M. Senevirathne
Dr. G.A.A.R. Perera
Dr. R.G.P.T. Jayasooriya
Ms. N.E. Wedamulla
Dr. Siyamalan Manivannan
Dr. Anantharajah Kaneswaran
Dr. Tharmarajah Thiruvaran
Dr. Balachandran Ketheesan
Dr. Sathiparan Navaratnarajah
Dr. K. S. Hemachandra
Dr. L.K. Weerasinghe
Dr. S. Dissanayake
Dr. Anushka U Rajapaksha
Dr. Tharanga Udagedara
Dr. Meththika Vithanage
Dr. Chamika Buddhinie
Dr. Nadeesh Adassooriya