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Editorial Policies

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Editor's Note

On behalf of the Editorial Board of the Journal of Dry Zone Agriculture (JDZA), I am delighted to present its 7th Volume (Number 1 and Number 2). JDZA is a peer-reviewed scientific journal aiming to publish up-to-date, high-quality original research articles related to all facets of dry zone agriculture. Six volumes of the JDZA have been published so far with the substantial contribution of many individuals, especially the authors and reviewers to the early development and success of the journal. Since the publication of the maiden volume, the editorial board of the JDZA constantly aspires to improve the quality and visibility of this journal. In this journey, initial steps are being taken to include the JDZA in online journal databases. Further, I am pleased to announce that, starting from the current volume (Volume 7), the JDZA operates under the terms of a Creative Commons license: Attribution 4.0 International (CC BY 4.0) which allows reusers to distribute, remix, adapt, and build upon the material in any medium or format, so long as attribution is given to the creator.

Agriculture is the mainstay of the Sri Lankan economy. Currently, the country is facing additional challenges due to COVID 19 pandemic together with the unpredictable climate changes which threaten the nation's food production profoundly. The research aiming to find innovative solutions to address these challenges has a key role to ensure food production sustainably. Despite the pandemic that intruded the scholarly works, the researchers are striving to discover solutions for the issues related to agriculture. As a result, thirty manuscripts were submitted to the Volume 7 of this journal. After preliminary screening and rigorous double-blind review, thirteen full-length research articles are published in this Volume. Needless to say that the findings published in this volume will contribute to improving the dry zone agriculture and related industries. I am certain that, upcoming volumes of this journal will carry more research articles in a diversity of perspectives related to dry zone agriculture.

I take this opportunity to extend my heartfelt gratitude to all who contributed immensely amidst the pandemic to bring out yet another volume of JDZA as per the schedule. My special thanks are due to the authors who submitted their works to the JDZA. I am especially indebted to the reviewers for sacrificing their time voluntarily to ensure the standard of the journal. Further, I would like to thank the Associate Editor and members of the Editorial Board for their relentless support to the release of this Volume of the JDZA possible. On the last note, I would like to express my sincere gratitude to the University of Jaffna for providing

partial financial assistance (University Research Grant - 2021) for the successful publication of this journal.

Best wishes and thank you in advance for your contribution to the upcoming volumes of JDZA.

Mrs. Subajiny Sivakanthan

Editor-in-Chief

3rd December 2021

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Rhizobia inhabiting *Clitoria ternatea* L. in Anuradhapura District, Sri Lanka: An assessment of stress tolerance and genetic diversity

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Abstract

Legume-Rhizobial symbiosis plays a greater agronomical and ecological significance as it provides fixed nitrogen through Biological Nitrogen Fixation. *Clitoria ternatea* is a leguminous plant that hosts a wide range of rhizobial strains. Even though it is a widely distributed plant, comprehensive information and studies conducted on the rhizobial - *C. ternatea* symbiosis is lacking in Sri Lanka. This study aimed to identify different stress-tolerant rhizobial strains inhabiting root nodules of *C. ternatea* growing in seven selected locations of Anuradhapura district of Sri Lanka. Twenty-eight pure rhizobial colonies were isolated and they were separately grown in ½ Lupin broths and were subjected to four different physiological conditions, pH, temperature, salinity, and drought. Most of the isolates were well-grown within the pH range of 5.0-8.0, the temperature range of 30-35 °C and at 0.2% Polyethylene glycol-8000

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(PEG) concentration. There was no observable pattern in the growth of rhizobial strains in different physiological conditions. The twelve rhizobial strains which showed high tolerance to extreme physiological conditions were subjected to a combination of physiological stress conditions of pH 8.0, temperature 36 °C, 3.0 % NaCl and 0.4 % PEG. The maximum growth in combination physiological study was observed in a strain collected at the Palugaswewa site. According to the dendrogram prepared by the Enterobacterial Repetitive Intergenic Consensus (ERIC) profile, twelve strains are genetically diverse, as they belonged to 11 clusters at 69.89 % of similarity level. These stress-tolerant rhizobial strains could be used for further studies on cross-inoculation of crop legumes as an alternative to the nitrogenous fertilizers.

Keywords: *C. ternatea*, ERIC, genetic diversity, rhizobium

INTRODUCTION

Nitrogen is an essential element for the growth and development of organisms. The earth's atmosphere contains about 78 % of nitrogen gas (Sur *et al.*, 2010) which is generally not reactive towards either oxidation or reduction. Therefore, most organisms are incapable of utilizing this nitrogen gas directly. However, a certain group of prokaryotes is capable to reduce this inert nitrogen into ammonia through a process known as Biological Nitrogen Fixation (BNF) (Santi *et al.*, 2013). BNF benefits both agriculture and the environment as it provides the nitrogen requirement of the plant and reduces the application of chemical fertilizers with nitrogen to the crop plants (Olivares *et al.*, 2013). BNF helps in reducing costs for farmers, improving soil quality and also mitigation of greenhouse gas emissions (Black *et al.*, 2012).

The prokaryotic rhizobia are important bacteria, involving in this process of BNF usually in leguminous plants root nodules. Most of the leguminous plants can fix atmospheric nitrogen by interacting with rhizobium and fulfill the nitrogen requirements. This symbiotic relationship helps the plant to grow well in nitrogen-deficient soils. The excess fixed nitrogen is released into the external environment when they die and that nitrogen can be utilized by other plants. Because of that, leguminous plants are extensively used as green manure in the agricultural fields. Hence, BNF is an important process in agriculture. The bacteria associated with the legumes are collectively known as rhizobia or root nodulating bacteria. Most of the current rhizobia belong to the Phylum proteobacteria which

includes mainly classes of Alpha and Beta proteobacteria. Thirteen genera of leguminous root nodulating species have been identified in the Class Alpha-Proteobacteria (Howieson and Dilworth, 2016).

Clitoria ternatea L. belongs to Family Fabaceae which is commonly known as “butterfly pea”, “Asian pigeon wings”, etc. These plants can be grown in different soil conditions where they can survive in soils with a pH range from 5.5- 8.5, including the calcareous soils. They can survive in prolonged droughts as well as in extended rainy seasons. *C. ternatea* has numerous medicinal values. Moreover, they are also grown as ornamental and fodder plants (Chukwuma *et al.*, 2014; Gupta *et al.*, 2010). This plant is a naturally growing abundant plant in the Anuradhapura district and it is usually grown as an ornamental plant.

Even though a limited number of studies have been conducted on the symbiosis between *C. ternatea* and rhizobia, several types of research have found that *C. ternatea* has a high potential to fix nitrogen through symbiosis with rhizobia (Oguis *et al.*, 2019). Different rhizobial strains have been isolated from *C. ternatea* growing in Thailand. They have been identified as *Bradyrhizobium elkanii*, and *Bradyrhizobium japonicum* inhabiting in the *C. ternatea*, growing in Thailand (Aeron *et al.*, 2015).

This study focuses on the identification of rhizobial strains which are adapted to extreme environmental conditions such as high salinity, high temperature, high drought conditions and extreme pH. These extreme stress-tolerant and genetically diverse rhizobial strains can be used for cross inoculation in crop plants that are grown in stress-full environments. When these stress-tolerant rhizobial strains are successfully cross-inoculated into the crop plants they fix atmospheric nitrogen and fulfill the nitrogen requirement of the crop plant. Some studies showed that cross inoculation of rhizobia to the crops has increased the crop yield and the plant growth than applying chemical fertilizers (Bhardwaj *et al.*, 2014). The use of environment-friendly fertilizer is becoming a trend due to many adverse effects caused by chemical fertilizers. If the stress-tolerant rhizobial strains are successfully cross-inoculated into crop plants, the application of chemical fertilizers would no more be required. The main objective of this study is to identify different stress-tolerant rhizobial strains from the root nodules of *C. ternatea* in the Anuradhapura district of Sri Lanka.

MATERIALS AND METHODS

Root nodules of *C. ternatea* were collected from randomly selected seven locations, Anuradhapura Urban area (AP), Thalawa (TH), Mihintale (MH), Medawachchiya (MW), Kahatagasdigiliya (KH), Palugaswewa (PG) and Thanthirimale (TM) of the Anuradhapura district, Sri Lanka. Four plants from each location were selected and one nodule from each plant was used to conduct the study. The root nodules were surface sterilized and cultured in ½ Lupin agar medium (Somasegaran and Hoben, 2012) using the crush method. Then the culture plates were kept in dark for 3-7 days at room temperature and twenty-eight pure rhizobial colonies were obtained after 3-5 times of subculturing. Naming of each isolated rhizobial colony was done based on the respective abbreviations of each site (e.g. AP-4 is the 4th sample obtained from the Anuradhapura urban site). Finally, the obtained pure cultures were used for the physiological characterizations.

Physiological tolerance

The isolated pure rhizobial colonies were grown in ½ lupin broths under different physiological conditions. The salt tolerance was assessed by growing the isolated 28 rhizobial strains in different NaCl concentrations (0.1, 1.0, 1.5, 2.0, 2.5 and 3.0 %). The pH tolerance was assessed by culturing the isolated pure rhizobial strains in ½ Lupin broths in different pH values, ranging from 3.0- 9.0 and the isolated 28 rhizobial colonies were grown in different Polyethylene-glycol (PEG-8000) concentrations (0.1, 0.2, 0.3 and 0.4 %) to assess the drought tolerance. The cultures were incubated in dark condition for 3 days at room temperature. The temperature tolerance was assessed by incubating all the isolated rhizobial strains under five different temperatures (25, 30, 35, 40 and 45 °C) in dark condition for three days. After three days, the absorbance was measured at 600 nm using spectrophotometer (Shimadzu-1800UV double-beam UV/ visible).

Combinational physiological test

The twelve-extreme stress-tolerant rhizobial strains were subjected for the combination physiological test where they were inoculated in ½ Lupin broths with, 3.0 % NaCl concentration, 0.4 % PEG concentration, and pH 8.0. These samples were incubated at 36 °C for 3 days in the dark. Then the absorbance was measured at 600 nm using a spectrophotometer. The obtained data was analyzed using Microsoft Excel and SAS 9.1.3 version.

DNA fingerprinting using ERIC sequences as primers

The twelve Rhizobial strains that showed high absorbance at extreme physiological conditions were used for the genomic DNA extraction. Genomic DNA of these stress-tolerant rhizobial strains used for the PCR amplification (Thermal cycler: Takara, Japan), using two primers of ERIC 1R and ERIC 2R. Initially, the PCR mixture was set at 94 °C for 5 minutes, followed by 35 cycles at 90 °C for 30 seconds. Then at 46 °C for one minute and the final extension was carried out at 70 °C for 10 minutes. The PCR amplicons visualized using Agarose gel electrophoresis. The obtained data were analyzed using MINITAB 14.0 software.

RESULTS AND DISCUSSION

pH tolerance of the rhizobial strains

The Figure 1 shows the growth of 28 rhizobial isolates at different pH values (3.0-9.0) in Anuradhapura urban area, Kahatagasdigiliya, Mihintale, Medawachchiya, Palugaswewa, Thalawa and Thanthirimale sites, respectively.

Generally, the optimum pH range for the growth of rhizobial strains found between 6.0-7.0 (Somasegaran and Hoben, 2012). However, most of the isolates in this study showed an optimum growth in the pH range of 5 to 8. pH of the soil in the Anuradhapura district varies between 4.5-8.0 (Renuka and Senavirathna, 2017). This proves that the isolated rhizobial strains from the root nodules of *C. ternatea* well adapted for the existing pH conditions of the soil in the Anuradhapura district. However, in this study, the tolerance of rhizobial strains tested at pH 9.0 and most of the isolates showed high tolerance even at pH 9.0. The AP-2 showed the highest growth at pH 9.0 among all the other isolates.

The AP-2, AP-3 and AP-4 of the Anuradhapura urban site showed a similar growth pattern from pH 5.0-9.0. The isolate AP-2 showed the highest tolerance to pH-9.0 in this site and it also showed a significant growth at pH 3.0 as well. The overall maximum growth at the site Kahatagasdigiliya was shown by the strain KH-2 at pH 6.0. However, almost all the strains of the Kahatagasdigiliya site showed lower growth at the pH range of 3.0-4.0. Moreover, the strain KH-4 showed the highest growth at pH 9.0. At the site of Mihintale, the strains MH-2 and MH-1 showed a significant highest growth at pH 6.0 and pH 7.0 respectively. Even though the strain MH-4

showed considerably lower growth from pH 6.0-9.0, it showed the highest growth at pH 3.0. The isolate MW-2 of the Medawachchiya site showed a significant plunge in its growth at pH 7.0 when comparing its growth at pH 6.0 and 8.0. The isolate MW-4 showed overall-higher growth in pH range of 3.0 to 8.0, except pH 7.0 and its growth has significantly dropped at pH 9.0. In the site Palugaswewa, the strain PG-2 showed the overall-highest growth at this site at pH 8.0. The isolates in the Thalawa site showed a differential growth pattern with the changing pH. All these results prove that there is no observable pattern in growth in rhizobial isolated with changing pH conditions. The sensitivity of rhizobia to different factors is different from rhizobium species to species (Dludlu *et al.*, 2017). Therefore, the differential growth pattern is observed with changing pH values. Moreover, rhizobium species such as *Mesorhizobium* can survive in a wide range of pH (pH 3.0- 10.0) (Dludlu *et al.*, 2017).

Generally, almost all of the rhizobial isolates showed poor growth in the pH range of 3-4. However, few strains showed a higher tolerance even at pH 3.0 (AP-2, MH-4, MW-4 and TM-3). Moreover, the AP-2 showed the highest tolerance at pH 3.0. This further proves that the rhizobial strains can survive in a wide range of pH values. The acid-tolerant rhizobial strains have several mechanisms to increase the internal pH. They increase their internal pH through the proton pump systems, glutamic acid decarboxylase, etc. (Lei *et al.*, 2011). Highly alkaline soils above pH 8.0 tend to be high in sodium chloride, bicarbonate, and borate levels, and are often associated with high salinity which diminishes nitrogen fixation (Nandanwar *et al.*, 2020). Highly acidic pH (1.0-2.0) was not considered as these conditions are usually not prevailing in natural soils in Sri Lanka (Moormann and Panabokke, 1961). Most of the strains have grown well at higher pH values than the lower pH values of 3.0 and 4.0.

Highly acidic pH affects the nodulation process in which the signal exchange between the host plant and the microsymbiont is disrupted and secretion of plant flavonoids is also decreased. These effects decrease the *Rhizobium nod* gene induction, restricts nod factors and nod metabolite excretion. The restriction of nod factor signaling affects the root hair deformation and root curling. The low pH also affects the attachment of *Rhizobium* on to root and colonization and finally, root nodule formation (Ferguson *et al.*, 2013).

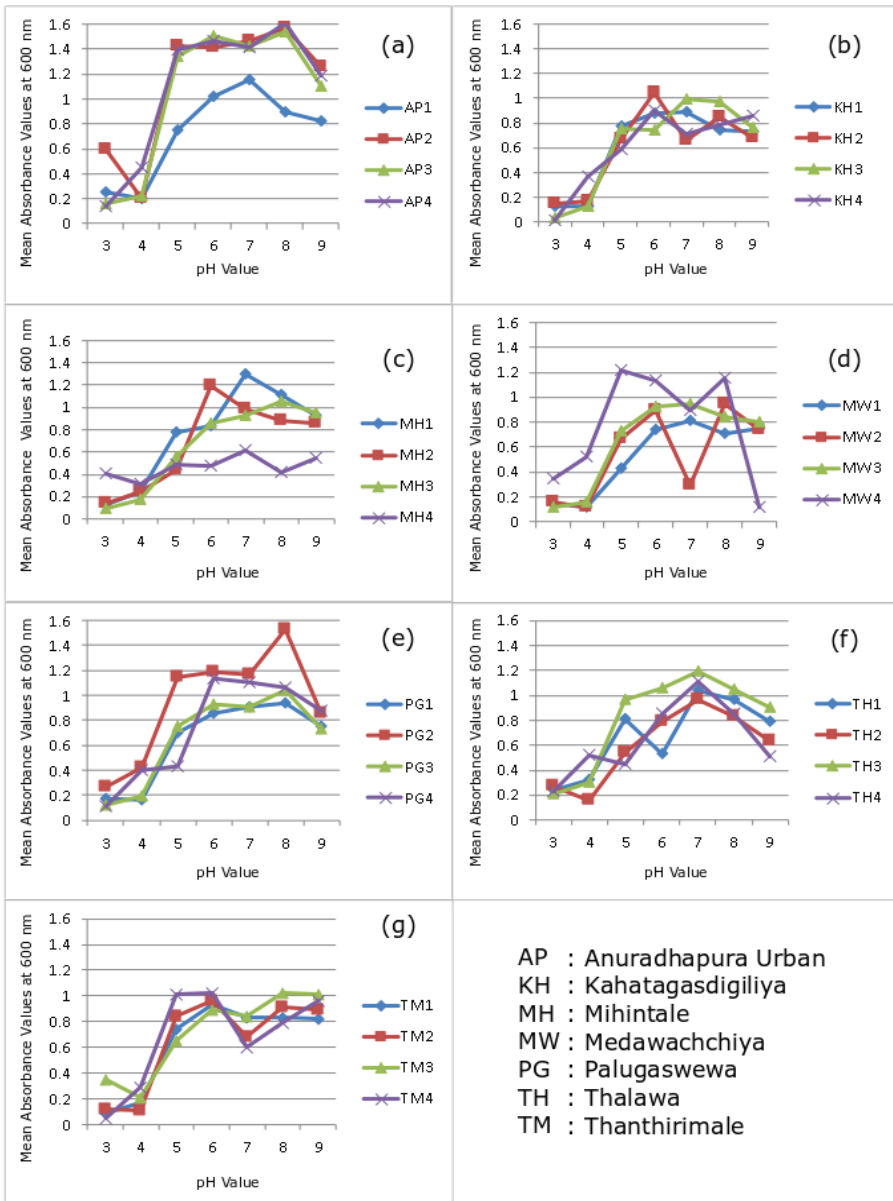


Figure 1: Graphs of (a) AP: Anuradhapura Urban (b) KH: Kahatagasdigiliya (c) MH: Mihintale (d) MW: Medawachchiya (e) PG: Palugaswewa (f) TH: Thalawa and (g) TM: Thanthirimale showing the growth of 28 rhizobial strains at different pH values (3.0 -9.0).

The rhizobial isolates that showed higher tolerance to pH 9.0 includes AP-1, AP-2, AP-3, AP-4, KH-4, MH-1, MH-2, MH-3, MW-3, PG-2, PG-4, TH-3, TM-4, TM-2 and TM-3.

Salinity tolerance of the rhizobial strains

The growth of the 28 rhizobial strains at different NaCl concentrations in Anuradhapura urban area, Kahatagasdigiliya, Mihintale, Medawachchiya, Palugaswewa, Thalawa and Thanthirimale sites are shown in the Figure 2. The graphs of the growth of twenty-eight rhizobial strains at different NaCl concentrations did not highlight a common pattern. The highest mean absorbance (~1.60 nm) value was observed in PG-2 at 1.0 % NaCl concentration while the lowest was observed in KH-3 at 1.5 % NaCl concentration. The rhizobial strains isolated from the Medawachchiya and Thanthirimale sites showed comparatively lower growth at different NaCl concentrations.

The strains at Anuradhapura urban site showed their maximum growth at 0.1 % NaCl concentration. The overall-highest growth at this site was shown by AP-2 at 0.1 % NaCl concentration. AP-2 showed maximum tolerance at 3.0 % NaCl concentration as well. The strains KH-2 and KH-3 of the Kahatagasdigiliya site showed their maximum growth at 1.0 % NaCl concentration. The strains KH-1 and KH-4 showed their maximum growth at 2.0 % and 0.1 % NaCl concentrations respectively. All the four strains in this site showed the lowest growth at 1.5 % NaCl concentration. MH-4 of the Mihintale site showed the highest growth at 1.0 % NaCl concentration in this site. In the site Medawachchiya, growth of all the strains has decreased gradually with the increasing salinity.

However, all the strains in this site showed their optimum growth at 0.1 % NaCl concentration. The strains PG-1, PG-3 and PG-4 of the Palugaswewa site showed their maximum growth at 0.1 % NaCl concentration while the PG-2 showed its maximum growth at 1.0 % NaCl concentration. The strain TH-4 of the Thalawa site showed the maximum growth at all salinity levels, except 0.1 % NaCl concentration. TH-3 of this site showed a significant plunge in its growth at 2.5 % NaCl concentration. But, its growth has considerably increased at 3.0 % NaCl concentration. In this site all the strains except TH-3 was well grown in 1.0 % NaCl concentration. TH-3 showed its highest growth at 1.5 % NaCl concentration. In the site Thanthirimale, the highest growth was shown by TM-2 at 1.0 % NaCl concentration. TM-1 and TM-3 showed their highest growth at 0.1 % NaCl

concentration. But, TM-2 and TM-4 showed their highest growth at 1.0 % NaCl concentration. Finally, these results suggest that, most of the strains grow well within the salinity range between 0.1 – 1.0 % but some prefer high salinity level as well.

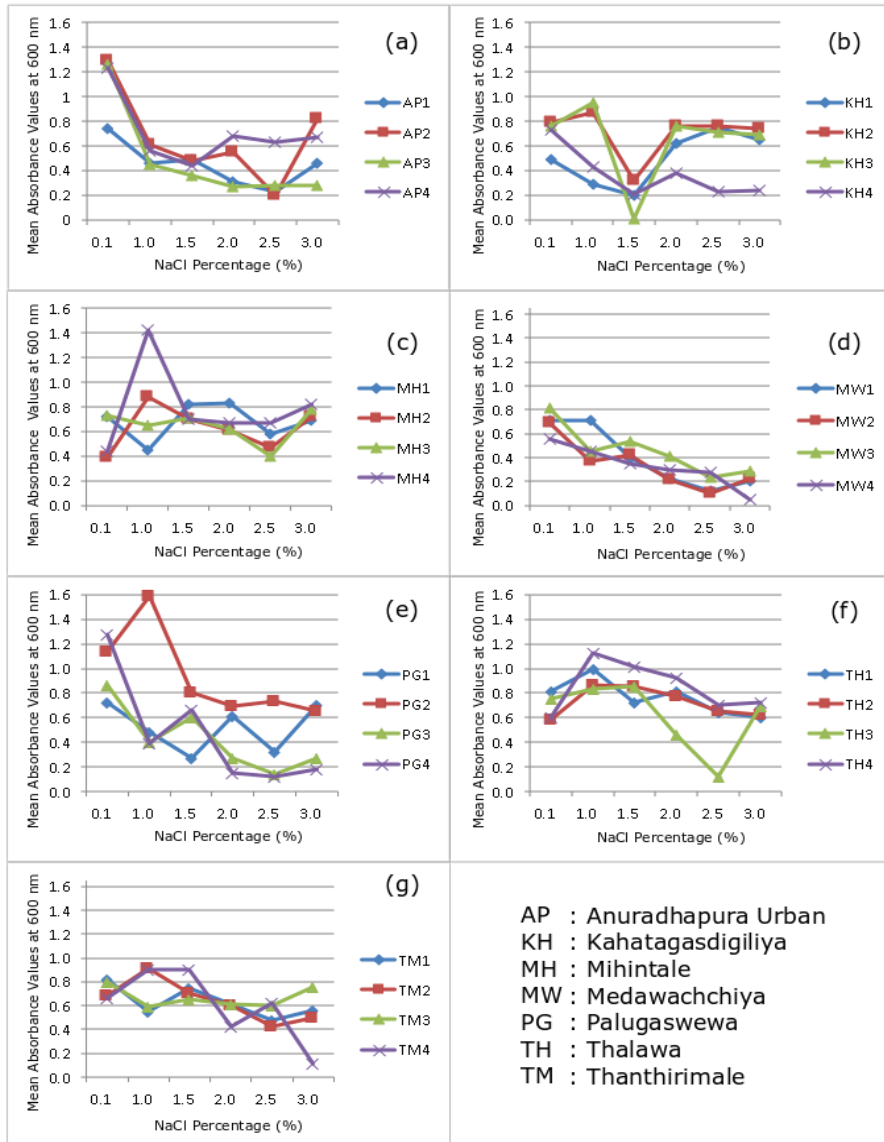


Figure 2: Graphs of (a) AP: Anuradhapura Urban (b) KH: Kahatagasdigiya (c) MH: Mihintale (d) MW: Medawachchiya (e) PG: Palugaswewa (f) TH: Thalawa and (g) TM: Thanthirimale showing the growth of 28 rhizobial strains at different NaCl concentrations.

The Anuradhapura district is in the dry zone where the temperature is high and the annual rainfall is low. This leads to elevated evaporation tending to accumulate high salt concentrations in the soil (Udupamunuwa *et al.*, 2020). Therefore, the soil of some areas of the Anuradhapura district experience high salinity level. Almost all the rhizobial strains isolated in this study showed a considerable growth even at 3.0 % salt conditions showing that the rhizobial strains are well adapted for the prevailing saline conditions of the soil in the studied areas of Anuradhapura district. Moreover, out of 28 isolated rhizobial strains seventeen strains (AP-2, AP-4, KH-1, KH-2, KH-3, MH-1, MH-2, MH-3, MH-4, PG-1, PG-2, TH-1, TH-2, TH-3, TH-4, and TM-3) showed highest tolerance to the extreme saline (3.0 %) conditions.

Temperature tolerance of the rhizobial strains

The growth of the 28 rhizobial strains at different temperatures in Anuradhapura urban area, Kahatagasdigiliya, Mihintale, Medawachchiya, Palugaswewa, Thalawa and Thanthirimale sites, are illustrated by the Figure 3.

In the Anuradhapura urban site, all four strains showed a comparatively higher growth from temperature 25 to 35 °C. However, their growth has drastically dropped from 40- 45 °C. But, the strains AP-1 and AP-2 showed a higher growth at 45 °C, than 40 °C. The overall-highest growth in this site was shown by AP-4 at 30 °C. The strain KH-1 of Kahatagasdigiliya site showed the overall highest growth at 25 °C. The other strains of this site showed their highest growth at temperatures of 30 °C and 35 °C. In the site Mihintale, MH-1, MH-2 and MH-3 showed their maximum growth at 30 °C while MH-4 showed their maximum growth at 35 °C. In the site Medawachchiya, MW-4 showed considerable higher growth from temperature 25- 35 °C. However, its growth has significantly dropped at temperatures of 40 °C – 45 °C, when compared to the other strains of this site. In the site Palugaswewa, PG-2 and PG-4 showed their highest growth at 35 °C while PG-1 and PG-3 showed their highest growth at 40 °C. In the sites of Thalawa and Thanthirimale, the overall-maximum growth was shown by TH-3 and TM-3 at temperatures of 35 °C and 30 °C respectively. Furthermore, the temperature sensitivity also varies between rhizobial strains and there is no common pattern in the growth of rhizobium strains with changing temperature.

The average annual temperature of the north-central province of Sri Lanka is 27.3 °C whilst the mean maximum is ranging from 26.2 to 33.9 °C. In some regions, the weekly maximum temperature reaches up to high values such as 39°C (Udupamunuwa *et al.*, 2020). The optimum

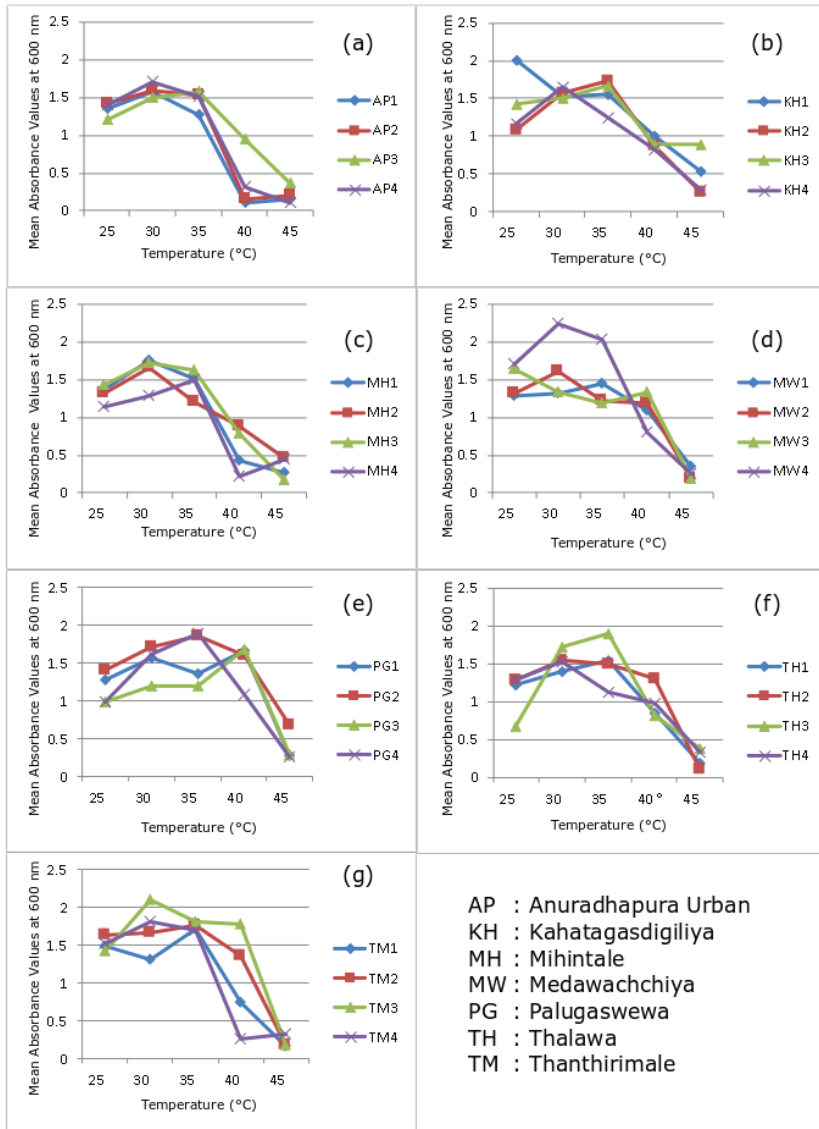


Figure 3: Graphs of (a) AP: Anuradhapura Urban (b) KH: Kahatagasdigiliya (c) MH: Mihintale (d) MW: Medawachchiya (e) PG: Palugaswewa (f) TH: Thalawa and (g) TM: Thanthirimale showing the growth of 28 rhizobial strains at different temperatures.

temperatures for the growth of most rhizobial strains are between 25 °C- 30 °C (Somasegaran and Hoben, 2012). The isolated strains also grew well in temperature between 25- 30 °C hence proving that evidence. Nevertheless, the highest growth was observed in 30 °C and 35 °C as well. However, most of the strains isolated in the Anuradhapura district grew well even at 40 and 45 °C temperatures. This is because the Anuradhapura district usually experience high temperature around 39 °C in some regions and the district is located in the dry zone which is prone to high drought conditions frequently (Kaleel and Nijamir, 2017).

Therefore, this suggests that most of the rhizobial strains are adapted to grow well in high temperatures. Moreover, most of the strains grow well even at 45 °C which was selected as the extreme temperature in this study. High temperature causes the interruption of signaling between the host plant and the rhizobium and also affects nodule development (Aranjuelo *et al.*, 2015).

The strains such as AP-3, KH-1, KH-3, MH-2, MH-4, MW-1, PG-2, TH-3, TH-4 and TM-4 showed the highest tolerance at 45 °C of temperature.

Drought tolerance of the rhizobial strains

The Figure 4 shows the growth of 28 rhizobial strains at different PEG concentrations in Anuradhapura urban area, Kahatagasdigiliya, Mihintale, Medawachchiya, Palugaswewa, Thalawa and Thanthirimale sites.

According to the current study all of the strains have grown well at 0.2 % PEG concentration. However, some strains have grown well even at 0.4 % PEG concentration, among them MH-3 was the best in survival.

In the Anuradhapura urban site, all the four strains showed their maximum growth at 0.2 % PEG concentration among them, AP-4 (~ 1.17 nm) showed the highest growth. The growth of all strains has reduced from 0.3 %- 0.4 % PEG concentration. All the strains of the Kahatagasdigiliya site also showed their maximum growth at 0.2 % PEG concentration. Thereafter, the growth of all the strains has gradually decreased. In the site Mihintale, MH-1 and MH-3 showed a considerable higher growth at 0.2 % PEG concentration, compared to the MH-2 and MH-4. All the strains in Mihintale site showed a gradual decrease in their growth after 0.2 % PEG concentration. However, the strain MH-3 showed a drastic plunge in its growth at 0.3 % PEG concentration and at 0.4 % PEG concentration

its growth has increased drastically. MW-3 of Medawachchiya site showed the overall-maximum growth at 0.2 % PEG concentration. PG-2 of the site Palugaswewa, TH-1 of Thalawa site and TM-1 of Thanthirimale sites showed the overall-highest growth in the respective site at 0.2 % PEG concentration. This proves that the 0.2 % PEG concentration is the optimum drought condition for the growth of isolated rhizobial strains in the Anuradhapura district. However, some strains are growing well even at 0.4 % PEG concentration. There was no clear pattern of growth observed in response to variation of PEG concentrations in the medium as in other physiological conditions.

Significant growth was shown by all 28 rhizobial strains under PEG-induced different drought conditions which varying the PEG concentration of growth medium from 0.1 % to 0.4 %. The PEG treatment results in the change in the osmotic potential in cells, there by stimulating the water-deficient conditions. Therefore, drought conditions are artificially induced in rhizobial cells. The water deficiency affects the symbiotic nitrogen fixation as well as the number of rhizobial strains in soil, their development and infection ability. The water deficiency causes the formation of free radicals resulting in protein denaturation and lipid peroxidation (Kibido *et al.*, 2019). The mean annual rainfall of the Anuradhapura district is 1368 mm (Climate-data.org). Nevertheless, during *Yala* season which lasts from the end of March to mid-May it receives about 300 mm of rain. As this district is located in the dry zone it experiences high evaporation leading to water scarcity, introducing drought conditions (Kaleel and Nijamir, 2017). The rhizobial growth has decreased with the increasing concentration of PEG which is supported by the study carried out by Udapamunuwa *et al.* (2020).

The strains AP-3, KH-4, MH-2, MH-3, MW-1, MW-2, PG-1, PG-2, PG-4, TH-1, TH-2, TH-3, TM-2 and TM-3 showed the highest survival at 0.4 % PEG concentration.

The tolerance of rhizobial strains under combination of physiological conditions

The growth of the 12 selected rhizobial strains in combination of different physiological conditions (pH 8.0, Salinity 3.0 %, Drought 0.4 %, and incubated at 36 °C) at 600 nm of optical absorbance are shown in the Figure 5. The stress-tolerant 12 rhizobial strains were subjected to the 'combination physiological conditions' in which the physiological

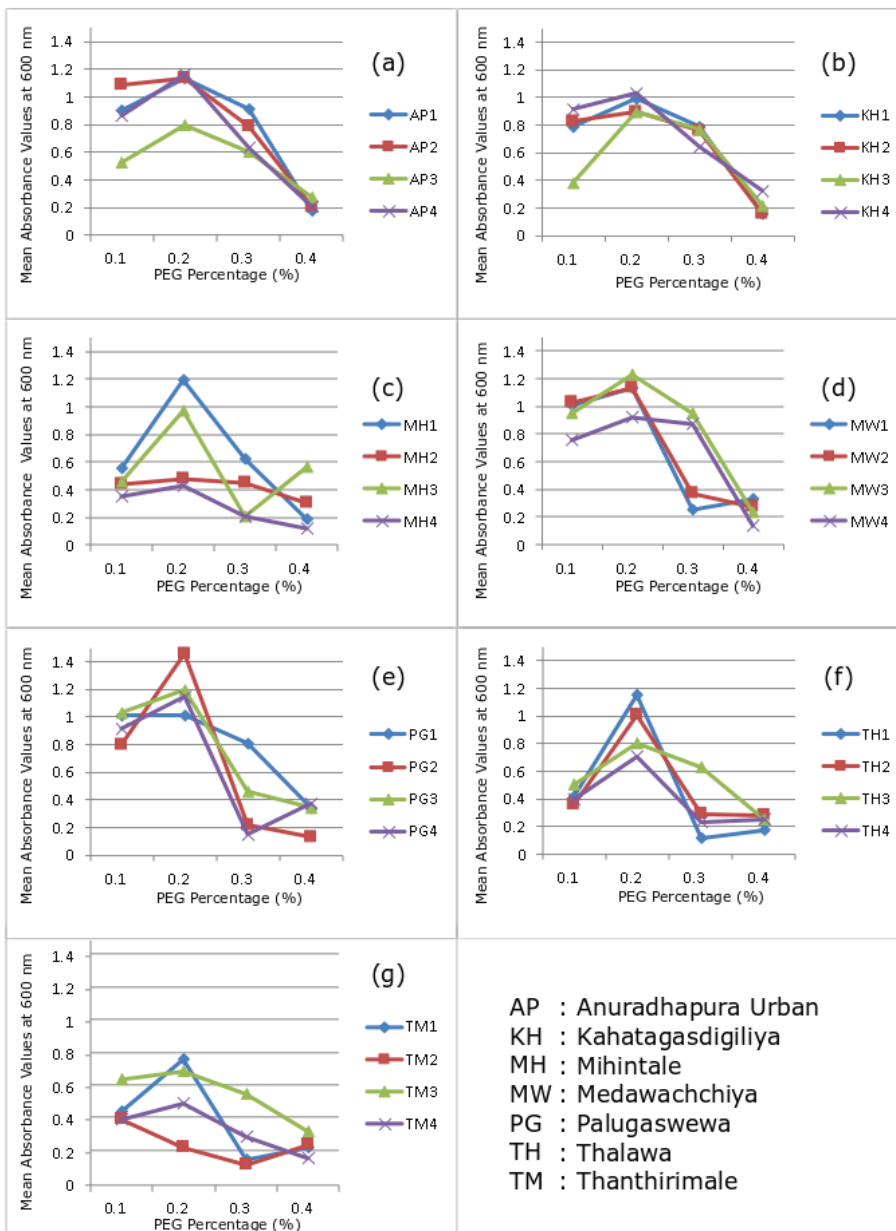


Figure 4: Graphs of (a) AP: Anuradhapura Urban (b) KH: Kahatagasdigiya (c) MH: Mihintale (d) MW: Medawachchiya (e) PG: Palugaswewa (f) TH: Thalawa and (g) TM: Thanthirimale showing the growth of 28 rhizobial strains at different PEG concentrations.

conditions were selected based on the prevailing natural environmental conditions in the Anuradhapura district. As the pH value of natural soil does not increase up to pH 9.0, pH 8.0 was selected for the combination study. Similarly, the strains were incubated at 36 °C as the natural soil temperature does not generally rise up to 45 °C. Due to the high-water deficiency and saline conditions of the dry zone and due to high evaporation, 0.4 % and 3.0 % PEG and NaCl concentrations were selected respectively. When these isolated strains were grown in a combination with extreme physiological conditions, some of them demonstrated much-reduced growth compared to the growth they have shown when testing for each physiological condition separately. The highest growth was observed in the strain PG-1. In addition to that, strain AP-2, AP-3, KH-1, KH-3, TH-4, and TM-2 showed considerably higher growth at these combined physiological conditions. The strains AP-4, KH-4, MH-3, MW-1, and PG-4 showed poor growth at these combined physiological conditions. Moreover, AP-4 showed the lowest growth among the poorly grown strains. The highest tolerance was observed in PG-1 where it showed maximum tolerance at 0.4 % PEG concentration and 3.0 % NaCl concentration. However, a high tolerance was not observed at 45 °C and pH 9.0. Further, PG-1 showed high tolerance when grown at 35 °C and pH 8.0 separately. Moreover, AP-4, KH-4, MH-3, MW-1 and PG-4 showed low growth at the combination of physiological conditions. The less growth of AP-4 may be due to the intolerability of 0.4 % drought condition as its growth is less at that particular drought condition. The growth of KH-4 and PG-4 is limited due to the intolerability of 3.0 % NaCl concentrations, as they showed poor growth when they were grown separately in that particular NaCl concentration. The poor growth of MH-5 has resulted due to intolerability of both pH and temperature.

Khalid *et al.* (2020) reported that the isolated rhizobial strains from the root nodules of *Arachis hypogaea* growing in an abiotic stress environment were well survived in the pH range of 5-10, salinity level 3.0 % (NaCl) and temperature range of 20 to 37 °C. We have also observed similar results as most of the isolated strains showed better survival at the same physiological conditions. Moreover, most of the strains isolated from *C. terantea* were well grown even in the extreme temperature of 45 °C.

Genetic diversity of isolated stress-tolerant rhizobial strains

The dendrogram with Average Linkage and Euclidean Distance is shown in the Figure 6. The ERIC profile which was used for assessing the genetic

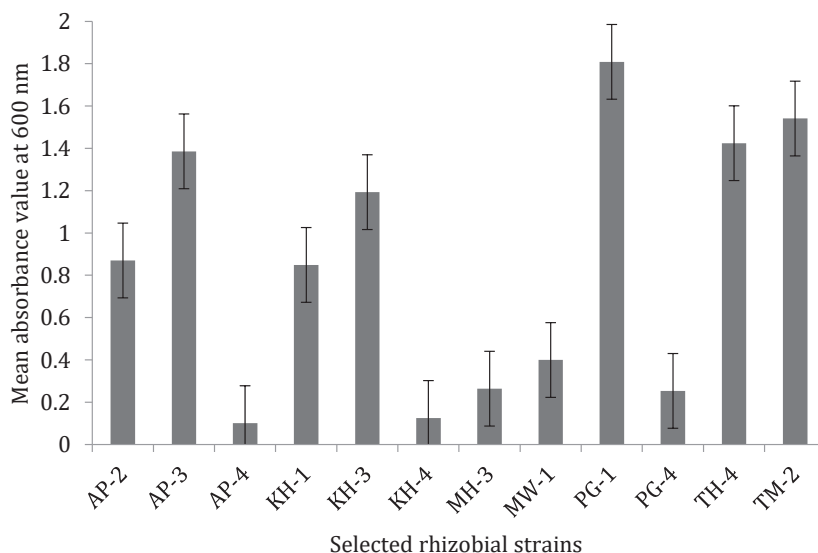


Figure 5: Growth of the 12 stress-tolerant rhizobial strains (PG-1^a, TM-2^a, TH-4^a, AP-3^a, KH-3^{a,b}, AP-2^{a,b,c}, KH-1^{a,b,c}, MW-1^{b,c}, MH-3^{b,c}, PG-4^{b,c}, KH-4^c, AP-4^c) in combination of different physiological conditions (pH=8.0, Salinity 3.0 %, Drought 0.4 %, and incubated at 36 °C) at 600 nm of optical absorbance (Means denoted by same letters are not significantly different at $p < 0.05$).

diversity showed a high polymorphism for the 12 selected rhizobial strains. The dendrogram was constructed from the polymorphic bands using Average linkage Euclidean distance method. The results suggest that strains MW-1 and MH-3 showed a closer relationship at 100 % similarity. Additionally, there was no significant difference between MW-1 and MH-3, which was supported by results of combination of physiological conditions study.

Moreover, Mihintale and Medawachchiya sites have more similar conditions, therefore microclimatic conditions are also similar showing a closer relationship between strains MW-1 and MH-3. At the 69.89 % similarity level eleven clusters were obtained in which two strains AP-2 and AP-3 of Anuradhapura urban site belong to two different clusters. However, the other selected strain (AP-4) in the Anuradhapura site does not belong to any of these clusters and it clusters with the strain (TH-4) isolated from the Thalawa site. The KH-3 and AP-2 clustered together at 69.89 % similarity further this is supported by the combined physiological

data which are rather similar. However, though PG-4 and AP-3 clustered together at 69.89 % similarity, their combined physiological data are not correlated. This is also because they are 30.11 % dissimilar to each other. Even though, AP-4 and TH-4 clustering together at 52.86 % similarity, their combined physiological data are totally different. This is because their genetic make-up is different. The strains isolated from the Palugaswewa site (PG-1 and PG-4) belong to different clusters at 52.86 % similarity. KH-1 and KH-4 strains isolated from the Kahatagasdigiliya sites also belong to two distant clusters. Therefore, these results suggest that there is no correlation between the location and the rhizobial strains. Furthermore, similar rhizobial strains can be found in different geographical locations. These results are further supported by the studies carried out by Udupamunuwa *et al.* (2020) and Samarakoon and Rajapakse (2020). Even though some of the strains belong to similar clusters, their

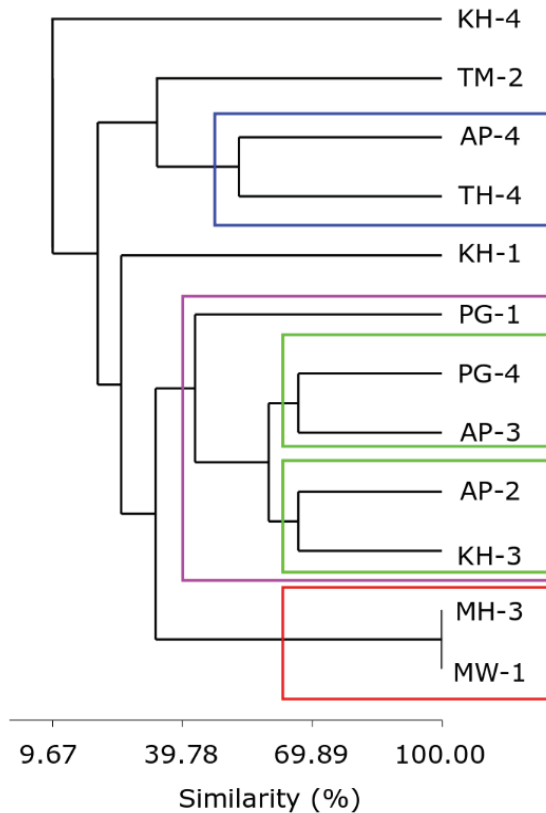


Figure 6: The Dendrogram with Average Linkage and Euclidean Distance.

physiological behaviors are different due to the difference in their genetic makeup. Furthermore, the ERIC profile suggests that all these 12 different strains are genetically diverse.

CONCLUSIONS

In this study, twelve rhizobial strains were identified, which showed high tolerance to extreme physiological conditions of temperature, salinity, drought, and pH. According to the dendrogram studies which was prepared using the ERIC profile, it was found that these twelve strains are highly genetically diverse, and they can withstand the extreme stress environmental conditions. PG-1 strain isolated from the Palugaswewa site showed the highest growth in combination of physiological study. Moreover, it is found that there is no correlation between the location and the rhizobial strains.

FUTURE DIRECTIONS

This study is very significant, as the isolated stress-tolerant rhizobial strains, in combination or as a single strain can be used for the cross inoculation for widely grown leguminous plants in Anuradhapura district. The successful stress-tolerant rhizobial strains can be used for manufacturing of Biofertilizers and the genetic information can be used for other strategies. Further studies can be focused for the identification of these isolated twelve rhizobial strains by sequencing of *16S rRNA* region and there is a high possibility for the identification of new rhizobial strains as well.

DECLARATION OF CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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Effect of silicon application on growth and yield of tomato (*Lycopersicon esculentum* Mill.) Var. Rajitha grown under water stress

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Abstract

This experiment was aimed to determine the effect of application of silicon on the growth and yield of tomato (var. Rajitha) under water stress condition. Treatments of the experiment were 75 mg of Si and no water stress (T1), 75 mg of Si and 50% water stress (T2), 150 mg of Si and no water stress (T3), 150 mg of Si, and 50% water stress (T4), no Si and no water stress (T5), and no Si and 50% water stress (T6). A pot experiment was conducted at a plant house in Horticultural Crop Research and Development Institute, Gannoruwa in 2019. The experiment was conducted as a Complete Randomized Design with a factorial treatment structure. There were 5 replicates in each treatment. Water stress and silicon were taken as main factors. Silicon was added as magnesium silicate and water stress was imposed by maintaining a moisture level equivalent to 50% of field capacity. According to the results, the significantly highest

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($p \leq 0.05$) mean height of plants (132.6 cm) in (13 weeks after planting) was observed in plants of T3 treatment, and the lowest mean height (107cm) was observed in plants of T6 treatment. Among the water stress treatments, T4 treatment reported significantly highest ($p \leq 0.05$) shoot/root ratio followed by T2 treatment. The application of magnesium silicate had improved yield parameters such as number of flowers, number of fruits, fruit weight, and fruit yield. In water stress treatments, T4 plants showed the highest flowering. Among the water stress treatment plants T4 resulted in the highest number of fruits per plant (13.6). Water stress negatively affects fruit weight but compared to T6 treatment plants (54.4 g) T2 treatment plants (55.7 g) and T4 treatment plants (56.3 g) showed higher fruit weight, but the treatments were not significantly different from each other. Lower fruit yield was observed in T6 treatment plants (0.707 kg) but T2 treatment plants (0.77 kg) and T4 treatment plants (0.967 kg) have shown higher fruit yield. The results of this experiment showed that the water stress reduced the growth of tomato plant and yield. But the application of magnesium silicate has a positive influence on growth and yield by enhancing the water tolerance of tomato under water stress conditions.

Keywords: growth parameters, nutritional parameters, silicon, tomato, water stress, yield parameters

INTRODUCTION

Global agricultural production is threatened by rapidly changing unpredictable climate conditions. Water deficiency is the most vital factor limiting agricultural activity in Sri Lanka. Temperature related extreme records have expanded over most areas in Sri Lanka. Yearly normal precipitation over Sri Lanka has been decreased throughout the previous 57 years at a rate of around 7 mm for each year. Temperature and water stress will have huge effects on soil moisture deficits and need additional irrigation water and water conservation is important in dry and intermediate zones in Sri Lanka for sustainable agricultural productivity (De Silva, 2006; Easwaran, 2018). Plant response to water stress involves different mechanisms ranging from stomatal closure, increase in root/shoot ratio, leaf area reduction, and osmotic adjustment. In the physiological mechanism of drought avoidance, maintenance of favorable water status in plants is achieved through either efficient stomatal regulation or high root activity (Kaya *et al.*, 2006). Tomato production in Sri Lanka is much lower than the world's normal as the regular climate

changes unfavorably influence normal production (Dishani and De Silva, 2016). Silicon (Si) is the second most abundant element in the earth crust after oxygen (Shi et al., 2016). Silicon is most commonly found in lithosphere in form of solution as silicic acid and all plant uptake directly as silicic acid (Ma et al., 2001). Si assumes an essential part in plant tolerance to environmental stresses. There are two types of resistance; (i) Stress avoidance-in the whole growth process does not meet with the face of adversity and (ii) Stress tolerance-plant has a capacity for environmental processes to remain normal. The effect of Si on the greater tolerance of higher plants to drought could be associated with an increase in the action of antioxidant defences, reduction in the oxidative damage to functional molecules and membranes, and maintenance of many physiological as well as photosynthetic processes under water stress conditions (Mauad et al., 2016). This study was conducted to investigate the effectiveness of Si in reducing the adverse effects of water stress and thereby increasing the growth and yield of tomato.

MATERIALS AND METHODS

Treatments and experimental design

A pot experiment was conducted for a period of 6 months at a plant house located in Horticultural Crop Research and Development Institute (HORDI), Peradeniya, Sri Lanka. As shown in Table 1, treatments of the experiment were 75 mg Si and no water stress (T1), 75 mg Si and 50% water stress (T2), 150 mg Si and no water stress (T3), 150 mg Si and 50% water stress (T4), no Si and no water stress (T5), and no Si and 50% water stress (T6). The experimental design was a Complete Randomized Design (CRD) with a factorial treatment structure. There are 6 treatments and 5 replicates. Stress and silicon were taken as factors. The total population is 30 plants.

Table 1: Treatments of the experiment

Treatment	Composition
T1	75 mg Si+ no water stress
T2	75 mg Si + water stress (50%)
T3	150 mg Si + no water stress
T4	150 mg Si + water stress (50%)
T5	No Si +No water stress
T6	No Si+ water stress (50%)

The plant house was maintained at a temperature of 28 °C with a thermostat and air circulation fans. Relative humidity was measured daily with a Relative Humidity meter. Recommended tomato seeds (var. Rajitha) was obtained from the vegetable division in HORDI. Silicon was added as MgSiO₃ (Magnesium silicate). According to the treatment order, magnesium silicate was added to the soil surface and mixed. The water stress plant root zone was covered using a transparent polyethylene sheet. Continue fertilizing tomato plants about every 3-4 weeks.

Water management

Water stress was imposed by maintaining a soil moisture level equivalent to 50% of field capacity, whereas the well-watered pots were maintained as control at full field capacity (100%) level. Field capacity was measured by volume basis. Plant available water for the water stress of 50% soil moisture deficit level was calculated by the difference between field capacity and permanent wilting point moisture content and divided by 2. The plant receives irrigation only when plant available water is depleted by 50% in water stress plants (Dishani and De Silva, 2016). The water-deficit treatments were applied for 3-week age tomato plants. Every day the water stress and plant water stress levels were measured using tensiometers.

Growth parameters

Plant height (cm) was recorded by using centimetre rod and the average height of tomato plant was calculated. The dry weight of the tomato plant shoots, and roots samples were determined separately in each replicate using a weighing balance. The samples were placed 60 °C for 72 hours (Mohammed *et al.*, 2018).

Yield parameters

Flowering was measured in each replicate at weekly interval and the average flowering of tomato plant was measured. The number of fruits per plant was recorded in each replicate at weekly interval and the average number of fruits of tomato plant was measured. Fruit weight (g) was measured in each replicate of tomato plants by using weighing balance and average fruit weight was measured. Fruit yield (kg) was measured in each replicate by using a weighing balance and average fruit yield was measured.

Statistical analysis

Data analysis was done by Analysis of Variance (ANOVA) and mean separation was done by LSD using appropriate SAS (University version) procedures.

RESULTS AND DISCUSSION

Growth parameters

Plant height

As shown in Figure 1, the significantly highest mean height of plants (132.6 cm) in (13 weeks after planting) was observed in plants with 150 mg Si and no water stress condition (T3) and the lowest mean height (107cm) was observed in plants with no silica and 50% water stress treatment (T6). These results indicate that the water stress affects significantly to reduce the vegetative growth of tomato plant. Water stress has several effects on tomato plant growth and the results of this experiment agree with the findings of Mohammed *et al.* (2018) that the tomato plants height and the number of leaves were reduced under different deficit irrigation levels. However, under water stress condition, Si added treatment showed higher plant height than that of no Si added treatment. Retarded plant growth and developmental processes are often observed in plants under water stress over time because photosynthesis and transpiration are inhibited

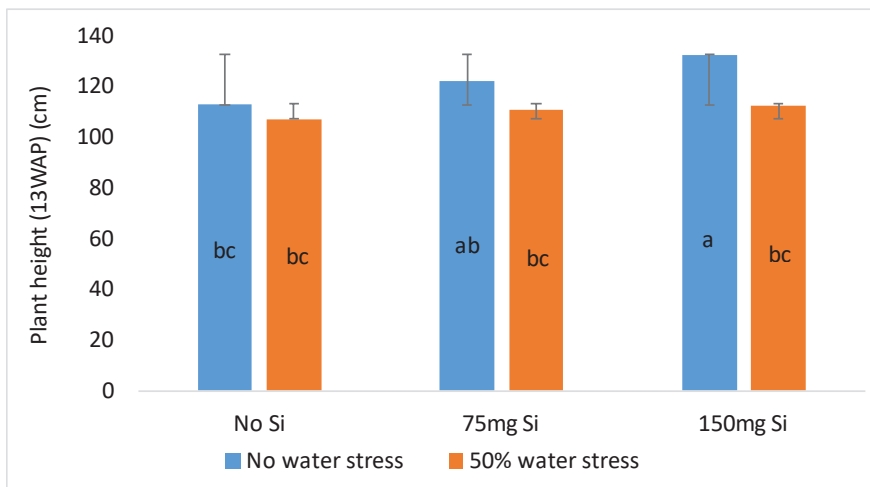


Figure 1: Effect of treatments on 13 Weeks after planting (WAP) plant height (letters inside the bar show the significance between treatments)

immediately after receiving the water stress (Sibomana *et al.*, 2013). However, study results revealed that the addition of Magnesium Silicate improved the water stress tolerance in tomato plants. Magnesium Silicate had a positive effect on growth because it might have been attributed due to increased photosynthetic activity of the plant, water metabolism, and membrane lipid peroxidation, more formation of carbohydrates, chlorophyll content, and increase enzymes under water stress condition, and more uptake of essential nutrients under the water stress condition.

Pulz (2008) found similar results by utilizing calcium and magnesium silicates in the place of dolomitic limestone which increased potato plant height. Similar results were also noticed by Dattatray (2018), Ullah *et al.* (2016) and Lu *et al.* (2016). Further, the Silicon applied plants have shown better performance in both water stress and no water stress condition. Silicon has great resistance to alleviate water stress and influenced tomato growth in addition to their effects on physiological characteristics.

Shoot / root ratio

As shown in Figure 2, the significantly highest ($p \leq 0.05$) shoot / root ratio of tomato was observed in 150 mg Si / no water stress treatments (T3) plants (6.4) followed by 75 mg Si / no water stress treatment (T1) plants (5.4) and then no Si / no water stress treatments (T6) plants (5.2). Among the water stress treatments, 150 mg Si treatment (T4) reported significantly highest shoot/root ratio followed by 75 mg Si (T2) and no Si treatments (T6). This ratio increased in plants with no water stress treatment but decreased in plants in water stress treatment. The results between water stress and no water stress are in line with the findings of Mohammed *et al.* (2018). However, no difference in shoot/root ratio was observed between the magnesium silicate applied plants and no silicon applied plants. The application of magnesium silicate increases plant growth in both water stress and no water stress treatments in this experiment. Similar results were noticed by Sandoval and Blanco (2017) in rice plant, the application of increasing doses of magnesium silicate significantly helped the growth variables compared to no silicon applied treatments, and similar observations were made by Dattatray (2018). Calcium Silicate and Potassium Silicate through modification of plant water relation stimulates sweet orange cell division and cell elongation boosts in plant immune system and enhances sweet orange plant growth.

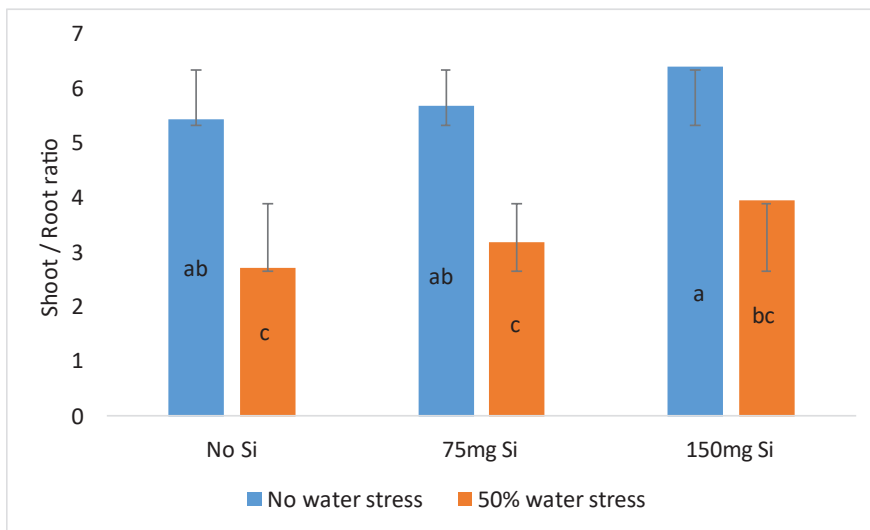


Figure 2: Effect of treatments on shoot/root ratio (letters inside the bar show the significance between treatments).

Yield parameters

Flowering in weeks after planting (WAP)

Flowering was observed in 5 WAP only in certain plants. Flowering data were collected from 5 WAP to 16 WAP. Early flowering was observed at 5 WAP in plants with water stress, no Si and from 8WAP to 13 WAP increasing in the flowering rate was observed. When considering the 11 WAP flowering mean values (Figure 3), they were significantly different between treatments. Significantly highest ($p \leq 0.05$) flowering showed in no water stress plants no Si (T5) and no water stress 75 mg Si (T1) plants. In water stress treatments 150 mg Si applied plants (T4) showed significantly highest flowering. Water stress badly affects flower formation. Results from this study agree with the finding of Olaniyi *et al.* (2010) in tomato plants. He reported that the water deficit stress increases the flower abortion. Water stress decreasing the tomato fruits settings, tomato fruit yield, and low-quality fruits might be due to the non-development of flowers. A similar result was observed by Sibomana (2013) as reported several changes in plant growth and developmental processes when plants are exposed to slow water stress over time because photosynthesis and transpiration are inhibited immediately after receiving the water stress. Water stress in the early growth period decreased the number of flowers

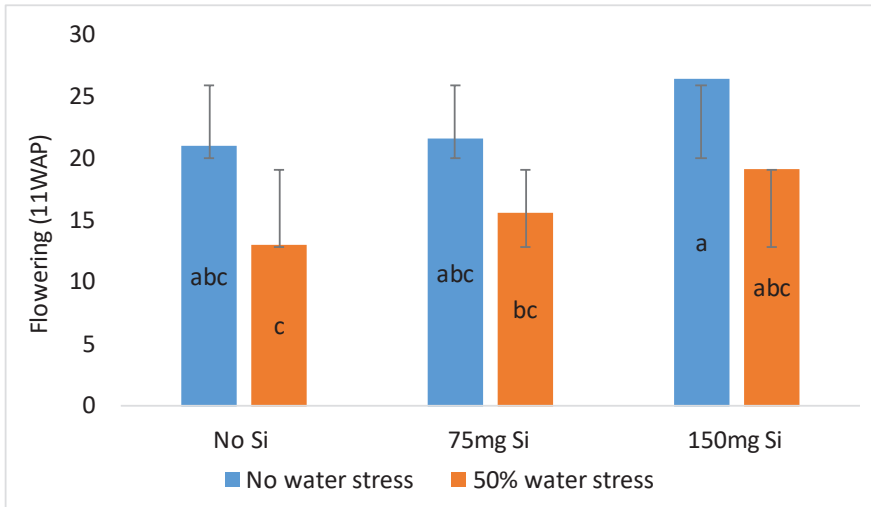


Figure 3: Effect of treatments on 11 weeks after planting (WAP) flowering (letters inside the bar show the significance between treatments)

leading to a reduction in the number of fruits and yield. However, in this experiment results showed (Figure 4) the application of magnesium silicate in water stress treatment has increased the flower formation than no Silicon applied water stress treatment plants.

Number of fruits per plant

The number of fruits per plant data was collected from 8 WAP to 16 WAP (Figure 4). Fruits started to appear in 8 WAP in several plants but 10 WAP fruits appeared in all plants. Results showed no water stress plant produced the highest number of fruit than the water stress plants. Based on the results significantly ($p \leq 0.05$) highest number of fruits was collected in no water stress 150 mg Si treatment plants (19.6), followed by no water stress 75 mg Si treatment plants (16.4) and no water stress no Si treatment plants (16). The lower number of fruits was collected in water stress no Si treatment plants (12), water stress 75 mg Si treatment plants (10.8), and water stress 150 mg Si treatment plants (13.6). However, among the water stress treatment plants 150 mg Si application treatment has shown significantly highest ($p \leq 0.05$) number of fruits per plant. These results indicate that water stress negatively affects fruit production and the results from this study are similar to those found by Mohammed (2018) in tomato plants. Jarosz (2014) indicates that silicon is the only element that does not harm plants when taken up in higher amount and

it improves plant fertilization, plant growth, and yield. Silicon application helped in improving fruit set and minimizing fruit drop of tomato as same as the result found by Dattatray (2018).

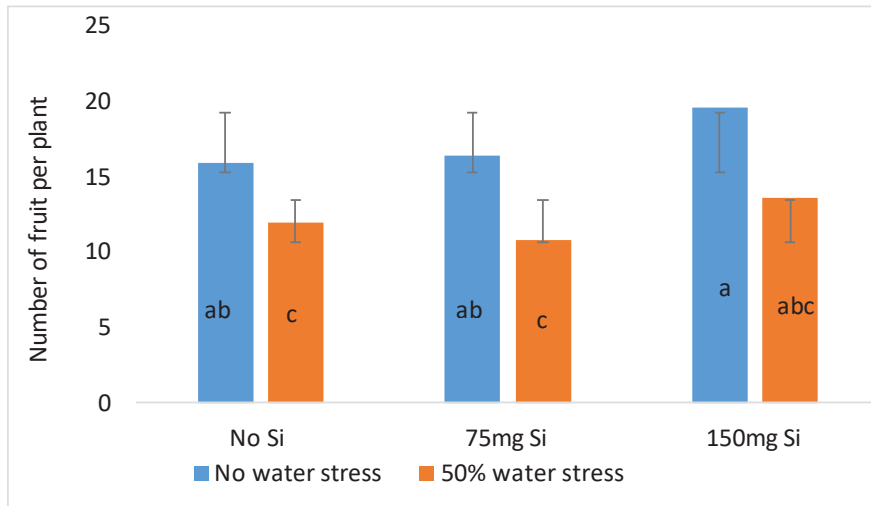


Figure 4: Effect of treatment on number of fruits per plant (letters inside the bar shows the significance between treatments).

Fruit weight

Results revealed that the average fruit weight was significantly different ($p \leq 0.05$) between water stress treatments and no water stress treatments (Figure 5). The highest fruit weight was recorded in no water stress 150 mg Si treatment plants (68.8 g), followed by no water stress 75 mg Si treatment plants (55.7 g), and no water stress no Si treatment plants (66.3 g). The lowest fruit weight was observed in water stress plants. Water stress negatively affect fruit weight but compared to water stress no Si treatment plants (54.4 g) 75 mg Si applied water stress treatment plants (55.7 g) and 150 mg Si applied water stress treatment plants (56.3 g) had higher fruit weight. But these treatments were not significantly different from each other. The tomato plant was most sensitive to water stress condition which reduced the average fruit weight in water stress conditions. The same result was found by Kamal (2013), who reported that the foliar application of potassium silicate improved average fruit weight and total yield of sweet pepper fruits and water use efficiency under water deficit irrigation level. Fruit weight is affected by water stress condition and fruit yield is reduced with a reduction in the amount of water uptake

from tomato plants. According to Caroline (2011) plants having sufficient water, form bigger fruits and at the same time get more nutrients under water supplied conditions, thus plants grow well and increase fruit yield and fruit quality. But in this experiment results indicate application of magnesium silicate had a positive effect of tomato fruit weight under water stress conditions. The same results were indicated by Dattatray (2018) that the foliar application of potassium silicate was found to be the best for improving the fruit weight and yield of sweet orange.

Fruit yield

According to the results, the highest fruit yield was reported in no water stress 150 mg Si treatment plants (1.4 kg) followed by no water stress no Si treatment plants (1.146 kg), and no water stress 75 mg Si treatment plants (1.173 kg). The lowest fruit yield was observed in water stress no Si treatment plants (0.707 kg). These results indicate that water stress negatively affects fruit yield.

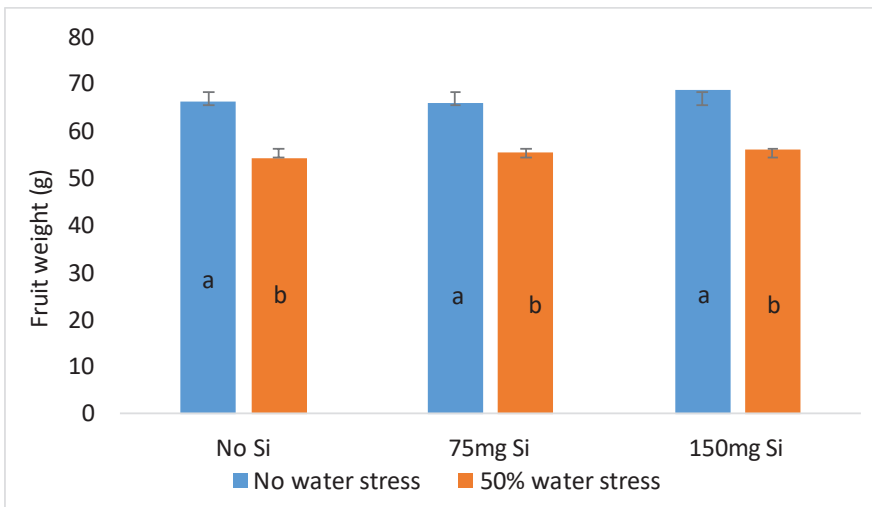


Figure 5: Effect of treatment on average fruit weight (letters inside the bar show the significance between treatments).

However, among the water stress treatment (Figure 6) significantly highest fruit yield was shown in 150 mg Si treatment plants (0.967 kg) followed by 75 mg Si treatment plants (0.77 kg). Results showed tomato plants reproductive processes were negatively affected by the water stress condition and water stress in the early growth period decreased the

number of flowers leading to a reduction in the number of fruits and yield. Caroline (2011) showed that the reduction in fruit weight and diameter under stress conditions may contribute to the reduction in fruit yield. And also, there was a reduction in the yield in all water stress treatment plants but magnesium silicate has increased the yield under water stress condition. Similar results were obtained by Jarosz (2014) that the higher total fruit yield in the treatments fertilized with the silicon enriched nutrient solution. Meena (2014) reported that the application of silicon fertilization to increased crop yield in tropical soils and observed that the silicon application may be one of the available resources for increased crop growth and crop yield in arid or semi-arid areas. Magnesium silicate may improve the other nutrient uptake because of that silicon applied plants have shown higher yield than other treatments. This study agrees with the findings of Lalithya et al. (2014) that at the time of fruit harvest minimum nutrients were available in the soil and maximum uptake of nutrients was shown in the silicon treated plants with maximum yield of sapota fruits.

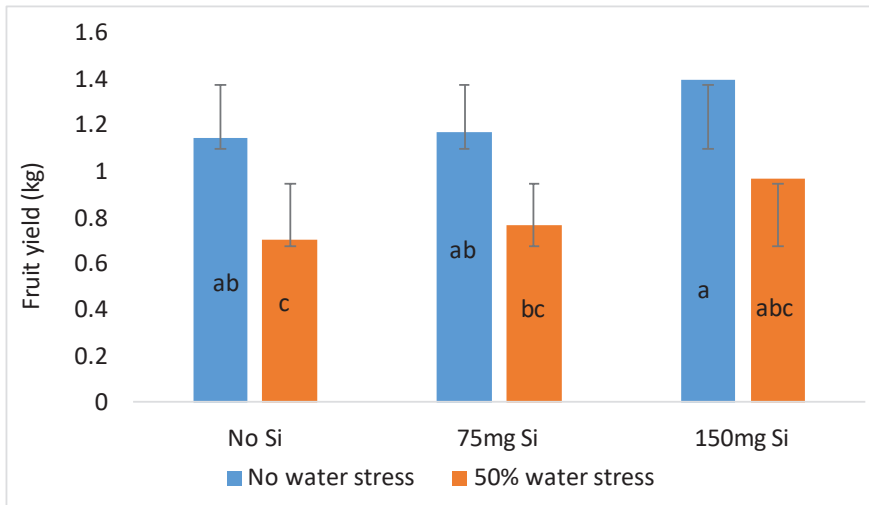


Figure 6: Effect of treatment on fruit yield (letters inside the bar show the significance between treatments).

CONCLUSIONS AND RECOMMENDATIONS

The findings of this experiment showed that the water stress causes a negative effect on the growth and yield parameters of tomato. Under moisture stress condition, a significant reduction of plant height, shoot/root ratio, flowering, number of fruits per plant, fruit weight, and fruit

yield were reported. However, the findings of the study showed that the application of magnesium silicate has a positive influence on growth and yield parameters on tomato var. Rajitha under the water stress conditions. Further experiments are needed to find the effect of silicon supplements because in tropical soils silicon application may be one of the available technologies to increase crop growth and yield in dry and intermediate zones of Sri Lanka.

DECLARATION OF CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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Evaluation of bran extracts of rice (*Oryza sativa*) and selected bean (*Phaseolus vulgaris* L.) varieties for their antioxidative and anti-hyperglycemic potentials

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Abstract

Search of therapeutic potential of natural and locally available food has become a trend due to increasing health concerns among consumers around the world. In this study, extracts of rice bran (*Oryza sativa* L.) and selected beans (red bean, red kidney bean, and white bean) (*Phaseolus vulgaris* L.) were obtained using 80% ethanol-water mixture to compare their anti-hyperglycaemic and anti-oxidative potentials. The total phenolic content (TPC), ferric reducing antioxidant power (FRAP), 2,2'-azino-bis (3-ethylbenzothiazoline-6- sulfonic acid (ABTS) radical scavenging activity, 2,2-diphenyl-1-picryl-hydrazylhydrate (DPPH) radical scavenging activity, and the carbohydrate hydrolyzing enzyme inhibitory potentials of the extracts were studied *in-vitro* using relevant assays. The highest phenolic content (0.122 mg of Gallic Acid Equivalent /g of extract) was

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found with red bean extract. FRAP values of the extracts were found to range from 48.98 to 75.94 $\mu\text{mol FeSO}_4/\text{g}$ bran extract. The highest ferric reducing power (75.94 $\mu\text{mol FeSO}_4/\text{g}$) was displayed by the bran extract of red kidney bean. The highest inhibitory effect against α -amylase (96.18%) was displayed by rice bran extract and the highest inhibitory effect against α -glucosidase (39.57%) was exhibited by red bean extract. This study concluded that the bran extracts of rice and the selected beans were potent sources of natural antioxidants and good postprandial hyperglycaemia regulators.

Keywords: Antioxidant activity, Antihyperglycemic activity, Rice bran, Diabetes

INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder that affects the metabolisms of carbohydrates, proteins, and lipids. As a chronic ailment, diabetes might affect an individual in numerous ways. Some of the serious health complications caused by diabetes are diabetic neuropathy, cardiovascular diseases, and retinopathy (Kumar *et al.*, 2012). The overall prevalence of diabetes mellitus in Malaysia was reported as 22.9% of the total population (Wan Nazaimoon *et al.*, 2013). Failure to arrest this rising trend in diabetes in the country can lead to serious social and economic problems. Diabetes can be generally categorized into several individual sub-types, but type I and type II are considered to be more prevalent worldwide (Agarwal and Gupta, 2016; Bhutkar and Bhise, 2012).

There is so much effort taken by countries around the world to address this severe health challenge. Strategies of treating diabetes include stimulation of insulin secretion, enhancement of the action of insulin at the target tissue and inhibition of the carbohydrate hydrolysing enzymes to control spiking of blood glucose (Funke and Melzing, 2006). Decrease of the postprandial hyperglycaemia has been identified as an effective therapeutic approach against non-insulin dependent diabetes. This is usually achieved by retarding the glucose absorption in the small intestine. The inhibition of carbohydrate hydrolyzing enzymes present in human digestive tract would play a significant role in this scenario (Bhutkar and Bhise, 2012). Pancreatic α -amylase catalyzes the initial hydrolysis of starch into oligosaccharides (Kandra, 2003; Tangphatsomruang *et al.*, 2005) while α -glucosidase which is a membrane-bound enzyme present in the small intestine will convert oligosaccharides to glucose (Abubakar

et al., 2017; Agarwal and Gupta, 2016). Moreover, the maintenance of the balance between free radicals and radical scavenging capacity of the body is also an important factor when coming to diabetes. Oxidative stress of a diabetic patient might also have the ability to get involved in the diabetic associated complication up to a certain extent (Yao *et al.*, 2010).

At present, evaluation of antidiabetic effect of natural plant sources has received much attention from researchers engaged in chronic ailments. It has been noted that the allopathic drugs used for treating diabetes might cause various gastrointestinal side effects (Bhutkar and Bhise, 2012). This claim has been debated by the research community repeatedly. According to the recent literature, seed coats of different beans and grains are reported to exert considerable anti-oxidative and anti-hyperglycaemic effects, which is beneficial for managing diabetes (Abubakar *et al.*, 2017; Adekola *et al.*, 2017). In this study, we aimed to evaluate the anti-oxidative properties and anti-hyperglycaemic potentials of the extracts of rice bran and seed coats of selected beans grown in Malaysia. Up-to-date, a study on the anti-hyperglycaemic potential of rice bran of CI220 hybrid type with different bean seed coats has rarely been investigated. The outcome of this kind of study might help to gain a broad idea with regard to the use of rice bran and different seed coats as precursors for developing functional foods to manage diabetes.

MATERIALS AND METHODS

Materials

Three samples of rice bran (CI220 hybrid type) were obtained from BERNAS Company Sungai Baru Complex, Alor Setar, Malaysia. Three samples of white bean, red kidney bean and red bean were obtained from a local supermarket in Semenyih, Malaysia. Sigma-Aldrich company, Malaysia supplied Porcine pancreatic α -amylase and α -glucosidase.

Preparation of extracts

In order to collect the seed coat extracts, all the bean types were soaked in water for 48 h and subjected to air drying. Rice bran was dried in the same form of purchased from the market. The dried bran of beans and rice were finely ground into powder form and extracted with 80% ethanol-water mixture. The extracts were concentrated using rotary evaporator and freeze-dried subsequently (Abubakar *et al.*, 2017).

Antioxidant properties evaluation

Determination of TPC

The TPC content of crude extracts were determined as reported by Singleton *et al.* (1999). Accurately, 20 μL of sample, 110 μL of 10% Folin-Ciocalteu reagent and 70 μL of 7.5% Na_2CO_3 was mixed in a 96-well microplate. After incubating the samples for 30 min at room temperature ($25 \pm 2^\circ\text{C}$), value of the absorbance at 765 nm was recorded. Gallic acid was used as the standard. TPC of the individual sample was expressed as mg gallic acid equivalents per 1 g dry weight of the crude extract.

Determination of FRAP

The method reported by Benzie and Szeto (1999) was used with slight modification to carry out the assay. In order to prepare FRAP reagent, acetate buffer (300 mM, pH 3.6), $\text{FeCl}_3 \cdot \text{H}_2\text{O}$ (20 μM), and TPTZ (10 mM) solution were mixed in the ratio of 10:1:1 and was heated at 37°C . A 20 μL sample extract was mixed with 30 μL acetate buffer, 150 μL FRAP reagent and incubated for 8 min at room temperature in a 96-well microplate. Then, the value of absorbance at 600 nm was recorded. The FRAP values were expressed as μmol of FeSO_4 / g of crude extract.

ABTS radical scavenging activity

The protocol described by Re *et al.* (1999) was used to carry out the assay in a 96-well microplate. A solution of 7.8 mM of ABTS in potassium persulphate was kept at 37°C in dark condition for 16 h to prepare a stable ABTS radical cation stock solution. A 40 μL of seven times diluted ABTS stock solution and 160 μL samples extracts were incubated at $25 \pm 2^\circ\text{C}$ for 10 min in 96 microplate. The value of absorbance was recorded at 734 nm for each sample. The results were expressed as Trolox equivalents antioxidant capacity in μmol of Trolox / g of crude extract.

Determination of DPPH activity

Determination of DPPH radical scavenging activity was carried out in a 96-well microplate in accordance with the protocol described by Blois (1958). A 125 μM of DPPH radical and 75 μM samples extracts were incubated for 15 min at $25 \pm 2^\circ\text{C}$ in a 96-well microplate. The absorbance was recorded at 517 nm. Results were expressed as μmol of Trolox / g of crude extract.

Enzyme inhibitory assays

Alpha-amylase inhibitory activity

This assay was carried out according to the protocol given by Bernfeld (1955) with a slight modification. Briefly, a 50 μL portion of samples extract (200 $\mu\text{g}/\text{mL}$), 50 μL of α -amylase enzyme (13 mg/mL), 40 μL of starch in 100 mM sodium acetate buffer (pH 6.0) were incubated at 40 $^{\circ}\text{C}$ for 15 min. Thereafter, 0.5 mL of DNS (3,5-dinitrosalicylic acid) reagent was added, placed in a boiling water bath for 5 min, and allowed to cool in ice. The value of absorbance of each sample was recorded at 540 nm. The control experiments were carried out following the same procedure by replacing the samples extracted with 50 μL of acetate buffer. Percentage inhibition of amylase activity of each sample was calculated according to the following equation:

$$\text{Percentage of Inhibition} = [(\delta A_c - \delta A_s) / \delta A_c] \times 100$$

Where, δA_c = Absorbance of control - Absorbance of control blank, δA_s = Absorbance of sample - Absorbance of sample blank

Alpha-glucosidase inhibitory activity

The assay was performed by adopting the protocol described by Matsui *et al.* (2001) with a slight modification. Briefly, 50 mU/mL of α -glucosidase enzyme, 4 mM p-nitrophenyl- β -D-glucopyranoside, 40 μL of samples solution (200 $\mu\text{g}/\text{mL}$) in the total reaction volume of 100 μL in sodium acetate buffer (50 mM, pH 5.8) were mixed in 96 micro-well plate followed by incubation for 30 min at 37 $^{\circ}\text{C}$. The reaction was then terminated by adding 50 μL of 0.1 M Na_2CO_3 . The value of absorbance at 405 nm was recorded for each sample. The percentage of glucosidase inhibitory activity of each sample was calculated using the following equation:

$$\text{Percentage of Inhibition} = [(\delta A_c - \delta A_s) / \delta A_c] \times 100$$

Where, δA_c = Absorbance of control - Absorbance of control blank, δA_s = Absorbance of sample - Absorbance of sample blank

Statistical analysis

For all measurements, triplicate data (n=3) were taken. IBM SPSS software package (version 21.0) was used to analyze the data adopting one-way analysis of variance (ANOVA). When *F* values were significant, mean differences were compared using Duncan's multiple range test at 5% level of probability.

RESULTS AND DISCUSSION

The yields of the bran extracts obtained from rice and selected beans are shown in Table 1. According to the data, the highest yield was observed for rice bran (5.01%) while the lowest was recorded for white bean (1.05%). The yield data were found to follow the ascending order of white bean < red bean < red kidney bean < rice.

Table 1: Yield (%) of bran extracts of rice and selected bean seed coats.

Sample	Initial weight (dry basis) (g)	Weight of extract (g)	Yield (%) (w/w)
Red bean	100.00	3.00	3.00
Red kidney bean	100.00	3.54	3.54
White bean	100.00	1.05	1.05
Rice	100.00	5.01	5.01

Total phenolic compounds

The TPC of the extracts of rice bran and selected beans are shown in Table 2. According to data in Table 2, the TPC of the bran extracts were varied from 0.026 ± 0.001 - 0.122 ± 0.004 mg of gallic acid equivalent (GAE)/g of bran extract. Although the TPC values were found in the ascending order of white bean < rice < red kidney bean < red bean, there was no significant difference ($p > 0.05$) between the TPC of red bean and red kidney bean. The low phenolic content of white bean extracts can be due to its less coloured seed coats (Madhujith and Shahidi, 2005). According to some previous studies, the colours of the bran of grains are imparted by phytochemicals present in them which are known to possess antioxidant properties (Abubakar *et al.*, 2017; Yao *et al.*, 2010). Adekola *et al.* (2017) reported that red kidney bean seed coat was found to contain the highest TPC compared to seed coats of red bean, black eyed pea, and black bean. However, the results of the current study showed that the TPC of red bean was slightly higher than that of red kidney bean. This variation in results could be due to the differences in sample preparation methods or extracting solvents, or even the geographical origin of the sample. Arab *et*

al. (2011) previously commented that the rice bran extract might have the presence of tocotrienols and tocopherols.

Table 2: TPC and FRAP values of bran extracts of rice and selected beans

Bran extract	Antioxidant property	
	TPC (mg gallic acid equivalent (GAE)/g bran extract)	FRAP ($\mu\text{mol FeSO}_4/\text{g}$ bran extract)
Red Bean	0.122 ± 0.004^a	74.260 ± 0.551^b
Red Kidney Bean	0.120 ± 0.001^a	75.943 ± 0.575^a
White Bean	0.026 ± 0.001^c	48.980 ± 0.235^d
Rice	0.049 ± 0.001^b	59.020 ± 0.551^c

Data represented as mean \pm standard deviation. The mean values within each column bearing different superscripts are significantly different at $p < 0.05$.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP values of seed coats of beans and rice bran are compared as shown in Table 2. According to the data, the highest FRAP value was displayed by red kidney bean ($75.943 \pm 0.575 \mu\text{mol FeSO}_4/\text{g}$) while the lowest value was shown by white bean ($48.980 \pm 0.235 \mu\text{mol FeSO}_4/\text{g}$). The values were found to follow the order of white bean < rice < red bean < red kidney bean while being significantly ($p < 0.05$) different from each other. Zuo and Chang (2014) reported that the ferric reducing power is affected by the presence of condensed tannins in a sample. Moreover, it is reported that the tannin contents of dry beans are found to vary according to the bean species and the colour of seed coats.

ABTS radical scavenging activity

Dose-dependent ABTS radical scavenging activity values of the extracts of rice bran and beans are compared as depicted in Table 3. The results obtained at $50 \mu\text{g}/\text{ml}$ and $100 \mu\text{g}/\text{mL}$ concentration levels of all bran extracts were significantly different ($p < 0.05$). However, no significant

difference ($p > 0.05$) was noticed between the results obtained for rice and white bean at 200 $\mu\text{g}/\text{mL}$ concentration level. At 50 $\mu\text{g}/\text{mL}$, the highest ABTS activity was exhibited by the extract of rice bran while the lowest activity was displayed by red bean. At 50 $\mu\text{g}/\text{mL}$ level, the activity could be aligned in the order of rice > white bean > red kidney bean > red bean. At 100 $\mu\text{g}/\text{mL}$ level, the highest activity was observed for white bean, while the lowest was observed for red bean. The ABTS values of the extracts tend to follow the descending order of white bean > rice > red kidney bean > red bean. At 200 $\mu\text{g}/\text{mL}$ level, both white bean and rice bran exhibited the highest ABTS activity while red bean showed the lowest activity. The ABTS values at 200 $\mu\text{g}/\text{mL}$ were found to follow the order of white bean \approx rice > red kidney bean > red bean. Overall, all bran extracts showed a dose-dependent radical scavenging activity for the ABTS assay.

Table 3: ABTS values of bran extracts of rice and selected beans

Bran extract	Dose-dependent ABTS radical scavenging activity		
	50 ($\mu\text{g}/\text{mL}$)	100 ($\mu\text{g}/\text{mL}$)	200 ($\mu\text{g}/\text{mL}$)
Red Bean	6.820 \pm 0.252 ^d	9.533 \pm 0.407 ^d	11.987 \pm 0.455 ^c
Red Kidney Bean	9.287 \pm 0.131 ^c	11.463 \pm 0.287 ^c	12.810 \pm 0.537 ^b
White Bean	36.450 \pm 0.476 ^b	47.05 \pm 0.440 ^a	49.423 \pm 0.296 ^a
Rice	39.027 \pm 0.839 ^a	42.647 \pm 0.488 ^b	49.317 \pm 0.415 ^a

Data represented as mean \pm standard deviation. The mean values within each column bearing different superscripts are significantly different at $p < 0.05$.

DPPH radical scavenging activity

A dose-dependent DPPH value of extracts of rice bran and beans is shown in Table 4. According to the data, both red kidney bean and red bean extracts did not display ant significant ($p > 0.05$) difference at the 50 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ concertation levels. Nevertheless, all the extracts displayed significant ($p < 0.05$) differences of each other at the 200 $\mu\text{g}/\text{mL}$ concentration level. The DPPH values of the bran extracts at 200 $\mu\text{g}/\text{mL}$ followed the order of white bean > rice > red bean > red kidney bean. However, white bean extract displayed the highest DPPH value at all levels.

Overall, the bran extracts showed a dose-dependent response to DPPH activity. Further, the exhibited values for DPPH assay were comparatively higher than the respective values obtained for the ABTS assay. Previously, Adekola *et al.* (2017) also reported higher DPPH values than ABTS values in the case of red bean and red kidney bean seed coats. However, according to another study, red kidney bean is reported to show higher ABTS scavenging activity than DPPH scavenging activity (Fahad *et al.*, 2014).

Table 4: DPPH values of bran extracts of rice and selected beans

Bran extract	Dose-dependent DPPH radical scavenging activity		
	50 ($\mu\text{g}/\text{mL}$)	100 ($\mu\text{g}/\text{mL}$)	200 ($\mu\text{g}/\text{mL}$)
Red Bean	12.333 \pm 0.722 ^c	15.903 \pm 0.856 ^c	22.200 \pm 0.716 ^c
Red Kidney Bean	12.467 \pm 0.492 ^c	15.373 \pm 0.290 ^c	20.700 \pm 0.542 ^d
White Bean	83.193 \pm 0.920 ^a	112.203 \pm 0.276 ^a	117.243 \pm 0.410 ^a
Rice	82.163 \pm 0.818 ^b	94.163 \pm 0.519 ^b	110.187 \pm 0.477 ^b

Data represented as mean \pm standard deviation. The mean values within each column bearing different superscripts are significantly different at $p < 0.05$.

According to some other studies, the phenolic compounds present in grains were greatly contributing to their antioxidant activity (Yao *et al.*, 2010). In the present study, however, the non-phenolic constituents present in the extracts might have largely contributed to the radical scavenging activities (Ojha *et al.*, 2019). This fact is further confirmed by two other studies where non-phenolics were claimed to display high antioxidant activities in foods such as pomegranate (Mekni *et al.*, 2013) and *Buddleja asiatica* (Mortada *et al.*, 2008). The selected items were reported to contain proteinaceous inhibitors of the alpha-amylase (Le Berre *et al.*, 1997; Yamada *et al.*, 2001). Moreover, they are reported to have anti-obesity and extensive anti-diabetes potentials (Wang *et al.*, 2011).

Inhibitory activity against alpha-amylase

Comparative inhibitory activity of the extracts of rice bran and selected beans against α -amylase is shown in Table 5. Percentage inhibitory activity

of the different extracts against α -amylase was found to range from 48.96% to 96.18% where rice bran showed the highest inhibitory effect while the white bean showed the lowest. In fact, significant ($p < 0.05$) differences were observed within the extracts and the order of the potency was found to be as rice bran > red bean > red kidney bean > white bean.

Table 5: Inhibitory activity (%) of bran extracts of rice and selected beans against Alpha-amylase and alpha-glucosidase

Bran extract	Anti-amylase and anti-glucosidase activity	
	α -Amylase inhibition (%)	α -Glucosidase inhibition (%)
Red bean	94.487 \pm 0.545 ^b	39.567 \pm 0.722 ^a
Red Kidney Bean	66.383 \pm 0.592 ^c	31.103 \pm 0.380 ^c
White Bean	48.957 \pm 0.792 ^d	34.573 \pm 0.983 ^b
Rice	96.180 \pm 0.551 ^a	11.373 \pm 0.617 ^d

Data represented as mean \pm standard deviation. The mean values within each column bearing different superscripts are significantly different at $p < 0.05$.

Inhibitory activity against alpha-glucosidase

Comparative inhibitory activity of the extracts of rice bran and selected beans against α -glucosidase is shown in Table 5. In fact, the percentage inhibitory activities were found to range from 11.37 to 39.57% where the highest percentage inhibitory activity was claimed for red bean while the lowest inhibitory activity was shown by rice bran. The α -glucosidase inhibitory activities of bran extracts were significantly ($p < 0.05$) different from each other, and the values tend to follow the order of red bean > white bean > red kidney bean > rice. Accumulated evidence from various studies showed that the coloured grains are rich in anthocyanins and reckoned as competitive α -glucosidase inhibitor due to their structural similarities to acarbose, which is a known α -glucosidase inhibitor (Yao *et al.*, 2010). The flavonoid contents in them could partly contribute to their inhibitory activities against the two carbohydrate hydrolyzing enzymes (Marikkar *et al.*, 2016). Further, Barret and Udani (2011) stated that anthocyanins present in red kidney bean have the potential to inhibit α -glucosidase

enzyme activity. The present study demonstrated that the extracts of rice bran and selected beans were found to exert a stronger inhibitory effect against α -amylase than α -glucosidase. Nonetheless, Adekola *et al.* (2017) previously reported that the α -glucosidase enzyme inhibitory potency of the seed coats of red bean and red kidney bean were higher than their respective α -amylase inhibitory activity. Moreover, they stated that the seed coat of red kidney bean had the higher α -amylase and α -glucosidase inhibitory activity than red bean seed coat. This variation in results could be due to multitude of factors such as differences in sampling, cultivars and geographical influences.

CONCLUSIONS

This study results showed that the bran extracts of rice and the selected beans can be identified as rich sources of phenolic compounds. They displayed differences in their antioxidant properties and inhibitory potency against α -amylase and α -glucosidase. Hence, the brans used in this study have the potential to be used as natural antioxidants which has the capability to control postprandial hyperglycaemia. Therefore, this study will help to broaden the use of these brans of seeds as novel functional foods targeting the management of postprandial hyper-glycaemia in diabetic patients.

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DECLARATION OF CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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Technical efficiency and its determinants: Paddy farming in Mahakanumulla cascade system

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Abstract

Paddy farming is predominantly practiced in the dry zone of Sri Lanka. Most paddy lands in the dry zone belong to traditional hydrological cascade systems known as Village Tank Cascade Systems (VTCS) and paddy lands receive irrigation water from the tanks in VTCS. Land is a scarce resource for agriculture in Sri Lanka. Therefore, it is important to achieve high efficiency in paddy farming to increase paddy production in the country. A paddy farm is technically efficient if it is producing the maximum output using the minimum quantities of inputs, such as labour, capital, and technology. This study examines technical efficiency of paddy farmers in Mahakanumulla VTCS and its determinants. The technical efficiency of the farmers is estimated using parametric frontier technique; the Stochastic Frontier Analysis (SFA). In the first stage of the analysis a production function is estimated using the Maximum Likelihood Estimator. In the second stage, an inefficiency model is estimated with plot size, age,

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experience, household size, full time farming, and land ownership status as determinants of technical inefficiency. According to the results, the average technical efficiency of paddy farmers in Mahakanumulla VTCS is 92.3%. Only the plot size, experience, and household size are positively related to technical inefficiency and significant at 10%. According to the study findings, the technical efficiency of paddy farmers in Mahakanumulla VTCS does not vary significantly from the most efficient farmer in the area. This could be due to the fact that in the VTCS, farmers are operating under more homogenous conditions. However, it does not indicate that paddy farmers in Mahakanumulla VTCS are highly productive as results only reveal the technical efficiency level of paddy farmers in comparison to the most efficient paddy farmer in the area. Therefore, it is important that productivity improvements are continuously carried out.

Keywords: inefficiency model, land tenure, paddy, stochastic frontier analysis, technical efficiency

INTRODUCTION

Rice is the staple food of Sri Lankans. There are approximately 1.8 million farm families engaged in paddy cultivation in Sri Lanka. Paddy is grown on nearly about 957,596 ha of land and production is about 4,592,056 MT with an average yield of 4,795 kg/ha (Department of Census and Statistics, 2019).

Today, one of the main issues faced by Sri Lanka as well as the world is food scarcity due to rapid population growth. In order to face the challenge of food scarcity, it is vital to have an efficient agricultural sector. Efficiency can be defined as the ability to produce a given level of output at the lowest cost (Abdulai and Huffman, 1998). Efficient paddy farming systems will not help only to reduce the food shortage; but also, to generate new employment opportunities and to earn more foreign exchange.

To increase the production efficiency, it is essential to find out the individual efficiency level of farmers who produce paddy using inputs significantly affecting paddy production such as seeds, fertilizers, weedicides, pesticides, labor, and machines. Further, there could be other factors that affect the efficiency level of farms such as the socio-demographic characteristics of the farmer, institutional characteristics, and policy changes. Historically one of the important institutional arrangements in paddy farming is land tenure arrangements. Even though traditional tenure arrangements are

not present today in their original form, we can observe different land ownership statuses which was the base for tenure arrangements. While some cultivate their own land, some others cultivate land obtained on lease, rent, and or permits. Depending on land ownership status, farmer's efforts could be different. Therefore, it is important to identify the effect of land ownership status on the technical efficiency of farms. Further, paddy land is subjected to fragmentation. While plot size gets smaller, they are dispersed geographically making managing them difficult by one farmer. Therefore, it is important to find out the effect of land ownership and plot size on the farm efficiency as this knowledge is important to the policymakers in suggesting changes to agricultural land policies in Sri Lanka to increase productivity. The dry zone of Sri Lanka does not receive enough rainfall for paddy cultivation in the *Yala* season. To facilitate paddy cultivation two seasons per year, the Village Tank Cascade Systems (VTCS) were introduced to the dry zone of Sri Lanka by ancient rulers. The VTCS ensured optimum water management and continuous paddy cultivation in both rainy as well as drought seasons (Geekiyanage and Pushpakumara, 2013). In this context, this research is carried out with the objectives of examining the average level of technical efficiencies of paddy farmers in Mahakanumulla VTCS, examining different determinants affecting the technical efficiency of farmers in Mahakanumulla VTCS and identifying the effect of land ownership status and plot size for the efficiency of paddy farmers in Mahakanumulla VTCS.

MATERIALS AND METHODS

Technical Efficiency can be explained as a ratio between observed output and the frontier output of the corresponding farmer. The simple meaning of technical efficiency is getting the maximum amount of output from a minimum amount of input utilization. Technical inefficiency can be explained as the amount or level of output that falls below the frontier output (Danso-Abbeam, 2015; Mabe *et al.*, 2018). In this study, SFA was employed to estimate the production function. The SFA is based on a production frontier in an econometric specification. In SFA, a nonnegative random component is included in the error term which is used as the measure of technical inefficiency (Meeusen and van Den Broeck, 1977).

The stochastic frontier output varies around the deterministic frontier because of the noise effect. In the case of farm A (Figure 1), the noise effect is positive. In consequence, the stochastic frontier output (q_A^*) lies above the deterministic frontier. However, since the inefficiency effect is greater

than the noise effect, the observed output (q_A) lies under the deterministic frontier (Figure 1). In the case of farm B, both the noise and the inefficiency effect are negative. Thus, the stochastic frontier output (q_B^*) and the observed output (q_B) both lie under the deterministic frontier. Empirically, the noise effect is equally distributed around the deterministic frontier while the inefficiency effect tends to lie below. The features of the model generalize to the multi-input, multi-output case (Coelli *et al.*, 2005).

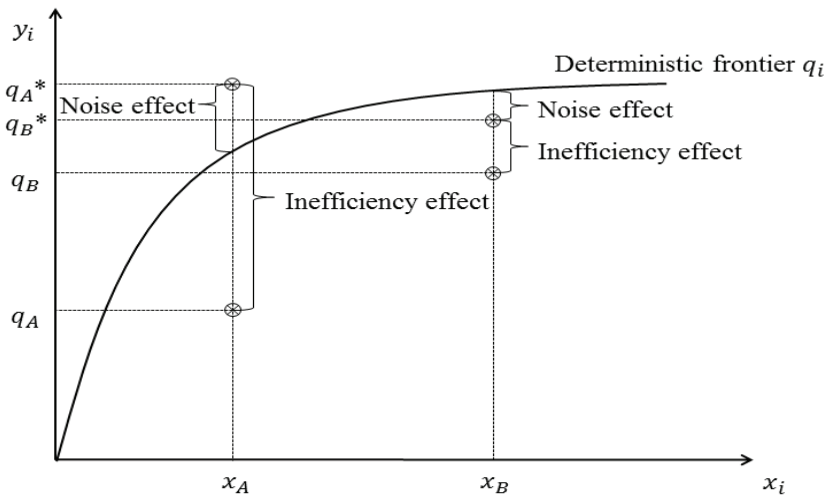


Figure 1: The Stochastic Frontier Model (Source: Coelli *et al.*, 2005)

The stochastic frontier production function is;

$$Y_i = f(x_i; \beta) + e_i$$

Where $i = 1, 2, 3, 4 \dots N$
 and $e_i = (v_i - u_i)$

Here, Y_i is the output level of the i^{th} farmer, $f(x_i; \beta)$ is the production function, x_i is the vector of inputs for the i^{th} farmer and a vector β is the parameters to be estimated. e_i is the error term which is consisted of two components as v_i and u_i . The error term v_i is for the random error as a result of measurement errors and other factors that are not under the control of farmers in production. u_i is a non-negative error term associated with farmer-specific factors, making farmers unable to obtain maximum efficiency in production. The u_i measures the technical inefficiency effects

that fall within the control of the decision-making unit.

According to the above description, the model in the log-linear form of the stochastic production frontier using the Cobb-Douglas functional specification introduced by Battese and Coelli (1995) which was used to determine the technical efficiency of the rice farmers is expressed below.

$$\ln Y_i = b_0 + b_1 \ln X_{1i} + b_2 \ln X_{2i} + b_3 \ln X_{3i} + b_4 \ln X_{4i} + b_5 \ln X_{5i} + (V_i - U_i) \quad (2)$$

Here $i = i^{\text{th}}$ farmer.

Y = Income from paddy (Rs./Year/Acre)

X_1 = Land preparation cost (Rs./Year/Acre)

X_2 = Seed cost (Rs./Year/Acre)

X_3 = Fertilizer cost (Rs./Year/Acre)

X_4 = Labour cost (Rs./Year/Acre)

X_5 = Total Harvest cost (Rs./Year/Acre)

TE of the i^{th} farmer can be specified as:

$$TE_i = \frac{\text{Observed output of } i^{\text{th}} \text{ farm household}}{\text{Frontier output of all farm household}} = \frac{Y_i}{Y_i^*} = \frac{f(x_i; \beta) \cdot e^{v_i - u_i}}{f(x_i; \beta) \cdot e^{v_i}} = e(-u) \quad (3)$$

$$\text{Technical inefficiency} = 1 - TE_i \quad (4)$$

(Konja *et al.*, 2019)

The error component v_i is assumed to be identically, independently and normally distributed with zero mean and constant variance, $N(0, \sigma^2)$. The error component u_i is also assumed to be distributed as truncation of a normal distribution with mean μ and variance σ^2 , such that the inefficiency error term can be explained by exogenous variables (Battese and Coelli, 1995).

The inefficiency distribution parameter can be specified as;

$$u_i = \delta_0 + Z_i \delta + \omega_i \quad (5)$$

Here Z_i is a vector of farmer characteristics and, δ is a vector of parameters to be estimated, and ω_i is unobservable random variables.

Determinants of technical inefficiency

Many studies have identified a positive relationship between technical efficiency and socio-demographic variable of the farmers, institutional characteristics and policy changes. The factors such as the age of farmer, farming experiences, plot size, and land ownership status were considered as determinants of technical efficiency in paddy farming in Mahakanumulla VTCS. Among different land ownership types, sole ownership, shared ownership, rented in, permits were considered in the inefficiency model (Ureta and Evenson, 2007).

The inefficiency model specified for Battese and Coelli (1995) specification was,

$$U_i = \alpha_0 + \alpha_1 \ln Z_1 + \alpha_2 \ln Z_2 + \alpha_3 \ln Z_3 + \alpha_4 Z_4 + \alpha_5 Z_5 + \alpha_6 Z_6 + \alpha_7 \ln Z_7 + \alpha_8 Z_8 + \epsilon \quad (6)$$

Z_1 = Plot size (acres)

Z_2 = Age of farmers (years)

Z_3 = Paddy farming experiences (years)

Z_4 = Sole ownership (sole ownership=1 if the land is solely owned and rented in=0)

Z_5 = Shared ownership (shared=1, rented in=0)

Z_6 = Permits (permits=1, rented in=0)

Z_7 = Household size

Z_8 = full-time farming (fulltime=1, parttime=0)

(Shantha *et al.*, 2012)

Data and sample

Individual paddy farmer belonging to the Mahakanumulla VTCS in the Thirappane Divisional Secretariat (DS) in Anuradhapura District was the unit of analysis. A cascade system is a hydrological system comprising of a network of tanks that harvest rainwater and a canal system that distributes water to the paddy lands. A cascade system was selected as the study site because it is a paddy-based production system and at present, the cascade system has undergone a lot of changes which may have affected paddy farming. For the sample, all farm households in the thirteen villages from the Thirappane DS division which belong to the Mahakanumulla VTCS were considered. Face to face questionnaire surveys was carried out during a ten-day period from the twenty-first of February to the first of

March in 2020. At the initial screening of data, incomplete questionnaires and outliers were removed. Data from a sample of 199 farmers were used for the analysis.

RESULTS AND DISCUSSION

Descriptive statistics

Summary statistics of the variables used in the stochastic production function estimation and the inefficiency model are given in Table 1. According to Table 1 data, the average size of paddy land per farm in the Mahakanumulla VTCS is 1.59 acres (6434.5 m²). This implies that the paddy farmers in the study area are mainly smallholder farmers operating on less than a hectare of paddyland.

Stochastic Frontier Production Efficiency Model

The maximum likelihood parameter estimates of the stochastic production function estimation are presented in Table 2. Coefficients, standard error, and *p*-value of each variable are shown.

The coefficients of all input costs are positive. Apart from labor cost and fertilizer cost, all the other inputs are significant at 5% significant level. This explains that output and hence income increases with the increased use of these variables. The cost of inorganic fertilizer is not significant due to less variability in the data. The government issues 50 kg of Urea, 16 kg of MOP, and 8 kg of TSP for one acre of paddy land free. Since most of the farmers in the Mahakanumulla VTCS are smallholders, paddy revenue may not be affected by the fertilizer cost.

Determinants of technical inefficiency of rice farmers

The inefficiency model was run with stochastic frontier inefficiency estimates. The inefficiency model estimates are provided in Table 3. None of the variables are significant at 95% confidence level. However, log of plot size is marginally significant ($p=0.05$) and positively related. This implies that inefficiency increases with plot size. That could be because when plot size is small it is easier to manage than managing a large plot. Since most farmers are relying on family labor, larger plots could be difficult to manage. Therefore, smaller paddy farms could be technically more efficient than larger farms. The coefficient estimate for farming experience is -1.8472 and it is marginally significant ($p=0.056$). This result

Table 1: Summary statistics

Variable	Mean	Maximum	Minimum	Standard deviation
Production Function				
Input variables				
Land preparation cost (Rs./Year/Acre)	14343	36500	0	7457.54
Seed cost (Rs./Year/Acre)	5229	30300	0	4601.79
Fertilizer cost (Rs./Year/Acre)	4049	22150	0	7117.22
Labour cost (Rs./Year/Acre)	1102	19200	0	2658.03
Harvesting cost (Rs./Year/Acre)	11557	30650	0	6127.80
Output				
Income (Rs./Year/Acre)	97656	680000	10750	66756.21
Inefficiency Model Determinants				
Plot size (Acres)	1.59	7	0.25	1.39
Age (Years)	50.75	80	22	12.12
Experience (Years)	24.58	60	1.5	13.65
Household size	6.81	18	2	2.78

(Source: Field Survey, 2020)

Table 2: Maximum-Likelihood Parameter Estimates for the Stochastic Production Frontier Model

Variable	Coefficient	Standard error	P-Value
<i>ln</i> land preparation cost	0.2007	0.0289	0.000
<i>ln</i> seed cost	0.0683	0.0213	0.001
<i>ln</i> fertilizer cost	0.0006	0.0142	0.961
<i>ln</i> labor cost	0.0016	0.0110	0.880
<i>ln</i> harvest cost	0.1606	0.0294	0.000

Table 3: Maximum Likelihood Estimates of the Determinants of Technical Inefficiency Model for paddy farms in Mahakanumulla VTCS

Variable	Coefficient	Standard error	p-Value
<i>ln</i> plot size	1.2171	0.2617	0.050
<i>ln</i> age	2.1327	1.3510	0.483
<i>ln</i> experience	-1.8472	0.4575	0.056
<i>ln</i> household size	2.9304	1.5328	0.056
Sole ownership(dummy)	-1.3173	1.0388	0.205
Shared ownership(dummy)	-3.1124	2.6734	0.244
Permits(dummy)	-4.4239	4.4921	0.206
Full time farming dummy	25.18	1249	0.984

implies that the higher the experience of the farmer, the lesser the level of inefficiency. Even though it was hypothesized that the land ownership status has a significant impact on technical inefficiency, the results show no significant effect of land ownership.

Technical efficiency distribution among farmers

The obtained technical efficiency estimates were categorized into efficiency ranges. Then the frequency of farms under each range and frequency percentages were calculated and are presented in the Table 4.

Table 4: Distribution of Technical Efficiency among Farmers

Range	Frequency	Percentage	Cumulative Percentage
0-0.19	1	0.5	0.5
0.2-0.299	0	0	0.5
0.3-0.399	0	0	0
0.4-0.499	1	0.5	1.5
0.5-0.599	0	0	1.5
0.6-0.699	2	1	2.5
0.7-0.799	9	4.5	7
0.8-0.899	42	21	28
0.9-1	144	72	100.00
Total Farms	199	100	
Mean	0.923		
Maximum	1		
Minimum	0.183		
Standard Deviation	0.1065		

(Source: Field survey, 2020)

According to the frequency distribution, the number of paddy farmers is in the range 90%-100% of efficiency is 144. Only 1.5% of farmers are below 50% efficiency. The average technical efficiency of paddy farmers is 0.923 or 92.3%. That means only 7.7% of efficiency needs to be improved in paddy farmers. While high average technical efficiency could imply that a large majority of paddy farmers are technically efficient, it could also imply low variation among paddy farmers in Mahakanumulla VTCS.

Technical efficiency distribution by inefficiency determinants

In this study, plot size, age, experience, and land ownership were used as the inefficiency determinants of the farmers. Average values of estimated efficiency levels for different levels of these variables are presented to better understand the variation in the data.

Plot size

Plot size was categorized into eight categories as shown in Table 5. The frequencies, percentages, and average efficiencies of each size category of paddy land are shown in Table 5. It could be seen that smaller land plots have a higher average level of technical efficiency compared to larger land plots.

Table 5: Plot size vs. average efficiency and frequency distribution

Plot size (Acres)	Frequency	Percentage	Average efficiency
<=1	118	59	0.943
1-2	47	24	0.922
2-3	17	8.5	0.883
3-4	4	2	0.925
4-5	7	3.5	0.889
5-6	4	3	0.776
6-7	2	1	0.811

(Source: Field survey, 2020)

Age of the farmer

The age of the farmer was categorized into six categories according to the age intervals as 20-29, 30-39 ...70-80. The frequencies, percentages, and average efficiencies of each category are displayed in Table 6. According to Table 6, the highest number of farmers belong to the age category of 50s. It is about 61 farmers or 30.5% of the sample. Even though not statistically significant some increase in the average technical efficiency with age could be observed. The highest efficient farmers are in the age category between 60-69.

Farming experience

Paddy farming experience was categorized into six categories as shown in Table 7. The frequencies, percentages, and average efficiencies of each category are shown in Table 7.

Table 6: Age of the farmer vs. average efficiency and frequency distribution

Age	Frequency	Percentage	Average efficiency
20-29	10	5	0.893
30-39	29	14.5	0.887
40-49	48	24	0.917
50-59	60	30.5	0.925
60-69	40	20	0.956
70-80	12	6	0.94

(Source: Field survey, 2020)

According to Table 7, 52 farmers or 26% of the sample have 20 to 29 years of experience in paddy farming. More than 45% of farmers are having more than 30 years of experience in paddy farming. The average efficiency shows an increase with the farming experience.

Table 7: Farming experience vs. average efficiency and frequency distribution

Experience	Frequency	Percentage	Average efficiency
0-9	36	18	0.876
10-19	30	15	0.894
20-29	52	26	0.927
30-39	43	21.6	0.958
40-49	27	13.5	0.95
50-60	11	5.5	0.935

(Source: Field survey, 2020)

Land ownership

Land tenure is categorized into three categories as sole ownership, shared ownership, permits, and rented in. The frequencies, percentages, and average efficiencies under each ownership category are displayed in Table 8.

Table 8: Land ownership status vs. average efficiency and frequency distribution

Tenure status	Frequency	Percentage	Average Efficiency
Sole ownership	148	74.5	0.928
Shared	21	10.5	0.966
Permit	5	2.5	0.954
Rented in	25	12.5	0.853

(Source: Field survey, 2020)

According to Table 8, 74.5% of farmers are sole owners of the paddy land they cultivate. The average efficiency does not vary much according to the land ownership status. However, the average technical efficiency in rented land is lower than other status of land ownership even though it is not statistically significant.

Household size

The household size of the paddy farmer was categorized into five categories as shown in Table 9 based on the number of family members. The frequencies, percentages, and average efficiencies under each household size category are displayed in (Table 9). According to Table 9, 53% of farmers have a family with 5-8 members. It may be that when family size is high, income generated from paddy farming is not sufficient. Therefore, farmers may engage in other occupations and hence may put less effort to paddy farming. It could also be that when family size is bigger, the family income from other sources may also be high. Therefore, farmers may exert less effort on paddy farming which results in a lower efficiency level.

Table 9: Household size vs. average efficiency and frequency distribution

Household size	Frequency	Percentage	Average efficiency
<=4 members	45	23	0.966
5-8 members	104	52.5	0.933
9-12 members	43	22	0.869
>13 members	6	3	0.772

(Source: Field survey, 2020)

CONCLUSIONS

The main objectives of this study were to assess the average level of technical efficiencies of paddy farmers in the Mahakanumulla VTCS and to identify determinants affecting technical efficiency. Among determinants of technical efficiency, the study was particularly interested in examining the effect of land ownership status and land plot size. The average level of technical efficiency among paddy farmers in the Mahakanumulla VTCS is very high at 92.3%.

Land plot size, age of the farmer, farming experience, household size and land ownership status were used in the inefficiency model. None of the variables were found to be statistically significant at 5% significance suggesting the homogenous nature of paddy farmers and farming in the Mahakanumulla VTCS. However, a slight variation in technical efficiency among paddy farmers could be observed suggesting mature, experienced farmers cultivating relatively small land plots and having small family sizes may be relatively more technically efficient than other paddy farmers in the Mahakanumulla VTCS.

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DECLARATION OF CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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Impact of government's motorcycle subsidy on job performance of agriculture extension officers in Southern province in Sri Lanka

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Abstract

Agricultural Extension is an ongoing, non-formal educational process that takes place over a period of time to transfer knowledge to rural farmers. In Sri Lanka, the ratio of farmers to extension officers is 800:1, and therefore, one extension officer has to extensively work with a larger group of farmers with a limited resource capacity. Considering these facts, the Sri Lankan government decided to provide motorcycle subsidy under National Budget Circular 2/2014 to increase the job performance and well-being of the Agricultural Inspectors. Even though the foresaid objective of this subsidy scheme is to improve the performances, there were no follow-up monitoring and evaluation system for this scheme. Therefore, this research is aimed at evaluating the impact of the government's motorcycle subsidy on job performance and well-being of Agricultural Inspectors in the southern province of Sri Lanka. The sample of 48 Agricultural Inspectors was randomly selected from 70 Agricultural Inspectors in the Galle district. Descriptive and inferential statistics tests

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were mainly used to analyze the data. Among the general constraints faced by the Agricultural Inspectors, the distance to be traveled, a higher number of farmers and area to be covered per day and less infrastructural facilities were major constraints. Importantly, the study revealed that the motorcycle subsidy has significantly affected the job performance of the Agricultural Inspectors directly by facilitating to perform their job roles and saving more time for Agricultural Inspectors to enhance their personal and career development through knowledge acquisitions.

Keywords: agricultural inspectors, job performance, motorcycle subsidy, well-being

INTRODUCTION

The agriculture sector plays a crucial role in Sri Lanka's economy by contributing 7.42% to the national Gross Domestic Product (GDP) and engaging approximately 23.73% of the workforce is related to the agricultural sector (Central Bank of Sri Lanka, 2019). However, agricultural production in developing countries continued to be low and it was generally believed that a dearth of information tailored to local needs and lack of technical knowledge at the farm level are the principal factors for this low and stagnant production (Muhammad, 1994). In that context, access to new knowledge and information plays a key role to improve the productivity agriculture sector. In this context, agricultural extension services play a bridging role between agriculture researchers who produced new knowledge and farmers (who use the new knowledge). Agricultural extension is an ongoing, non-formal educational process that assists in improving the living conditions of farmers and their family members by increasing the profitability of their farming activities. Therefore, extension service improves the farmer's knowledge, skills, and change of their attitudes in agricultural technology, farming activities, and agricultural marketing (Mahaliyanarachchi, 2003).

However, in most developing countries, there are few extension officers to serve many farmers and it is ranging from 1:100 to 1:10000 (Davis and Franzel, 2018). In Sri Lanka, the ratio of farmers to extension officer is 800:1. There is a need to determine the ways in which the same few extension officers can serve a larger group of farmers with minimum effort (Iwoga *et al.*, 2011). At this current scenario, extension services should contribute to increasing the productivity of the farming business and are supposed to support, guide, and direct farmers with the minimum

resource capacity. In the current context, agricultural extension services in Sri Lanka have been assigned to the authorities of the Department of Agriculture to ensure better performances with maximum efficiency. In order to obtain these services from agricultural extension officers, it is vital to motivate them to provide all the necessary infrastructure facilities and adequate staff. Furthermore, Mwangi and McCaslin (1995) point out that, extension workers who work harder will perform better if they are motivated and satisfied with their jobs. In addition, Lindner and Dooley (2002) note that effective performance in skills requires the application of related knowledge and helps make possible the acquisition of new knowledge. In addition, extension agents also need competencies in program planning and development (Boyd, 2004).

Considering these needs and requirements, Sri Lankan Government decided to motivate extension officers with the provision of the motorcycle subsidy under National Budget Circular 2/2014 to improve the job performance and well-being of the Agricultural Inspectors. Under this circular government provided the motorcycles to public officers engaged in field level duties, with the aim of expediting public service delivery and thereby providing their service more effectively to the public. However, there were no any formal mechanisms or procedures to evaluate the effectiveness of this subsidy program and therefore, the government does not have any procedure to continue this program.

Therefore, the main objective of this research is to analyze the impact of the government's motorcycle subsidy on job performance and well-being of Agricultural Inspectors and subsequently to evaluate the existing constraints of the Agricultural Inspectors of Sri Lanka. It is assumed that when Agricultural Inspectors are satisfied with their job and facilities, they would give a higher effort to complete their job tasks, and ultimately it helps to improve their overall performance.

Therefore, this research study will answer the following research questions; What are the general constraints of extension officers have to face? What are the direct impacts of Motorcycle subsidy on job performance and well-being of extension officers? Accordingly, this study is aimed to achieve main three specific objectives; to identify general constraints of extension officers' job performance and their knowledge dissemination and to study the direct impacts of Motorcycle subsidy on job performance, and to evaluate the impact of motorcycle subsidy on the well-being of extension officers.

MATERIALS AND METHODS

This study aims to analyze the impact of the government's motorcycle subsidy scheme using Agricultural Inspectors who are currently working in the three sub-agricultural divisions (East, Central, and West) in the Galle district. This research has been designed to collect data using a semi-structured questionnaire with the 48 Agricultural Inspectors which has been selected using simple random sampling out of 70 populations. Secondary data were gathered from previous articles, journals, government publications.

First, the research aims to identify the constraints faced by agricultural extension workers including the availability of logistics and other support, technical know-how, and other basic infrastructure facilities. Aligning to that, this study tries to see constraining factors associated with the covering area of Agricultural Inspectors, number of farmers' extension/visit per day, travel cost per day, travel time for extension work, availability of technical knowledge, adaptability to new knowledge and technology, the sufficiency of knowledge/skills available with Agricultural Inspectors, support from the farmers for the extension and the language barriers in the working area.

Furthermore, there are 13 variables were used to analyze the impact of motorcycle subsidy on the job performances of the Agricultural inspectors. Those variables were used to see any significant impact has happened on the improvement of the job role and job performances, covering a greater land area, accessing a higher number of farmers, improvement of the relationship with co-workers and farmers, improvement of the interest towards the job and the time for knowledge dissemination. The average score of the respondent was used to analyze the impact measures on the aforementioned variables.

Similarly, this study has focused to analyzing the impact of subsidy scheme on the different dimensions of the wellbeing of the Agricultural Inspectors using 6 variables. Those variables have been used to analyze the impact of subsidy scheme on personal security when travelling, balancing work-family life, saving time for knowledge enhancement, adequate time for leisure activities, time-saving for social interactions, and impact on health status.

RESULTS AND DISCUSSION

Prevailing constraints on the job performances of Agricultural Inspectors

In this study, selected few factors were evaluated as the constraining factors for job performances of the Agricultural Inspectors and how they have been perceived those factors as the constraining factors.

Covering area of Agricultural Inspectors

According to research findings, the majority of Agricultural Inspectors (76%) have to cover over 10 km² area per day while about 21% are covering 5-10 km² area and the rest of 3% are covering an area below 5 km² per day to cover their field work. Accordingly, Agricultural Inspectors need to cover a vast area within a limited time period and are perceived as one of the major constraining factors.

The number of farmers visits per day

Another important observed constraining factor was the number of farmers visit per day. According to Figure 1, 58% of Agricultural Inspectors visit more than 10 farmers per day, and 39% of Agricultural Inspectors visit between five to ten farmers per day while 3% visit less than 5 farmers per day. These findings imply the workload of Agricultural Inspectors per day with the minimum infrastructure facilities in some areas.

Travel cost per day

Another important constraining factor which was considered in this study is to travel cost per day which has to bore by the Agricultural Inspectors using their own money. According to the research, the majority (71%) of respondents spent over 100 rupees per day for the traveling cost while 29% spent 20-100 rupees per day. Moreover, Agricultural Inspectors perceived this traveling cost has to be recovered by any other incentives.

Travel time for extension work

Similarly, traveling time for the farmer visit has been considered as another constraining factor. According to the Figure 2, more than 60% of Agricultural Inspectors spend over 60 minutes for travelling to extension

workplace daily. While 34% spend 30-60 minutes for travelling to the extension workplace. Only about 5% of Agricultural Inspectors in the sample spend less than 30 minutes to travel.

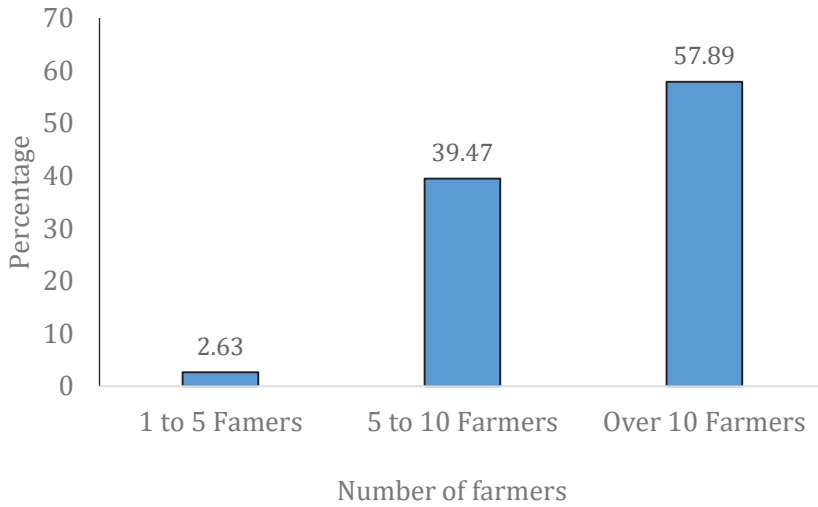


Figure 1: Number of farmers visit per day (Source: Survey data, 2020)

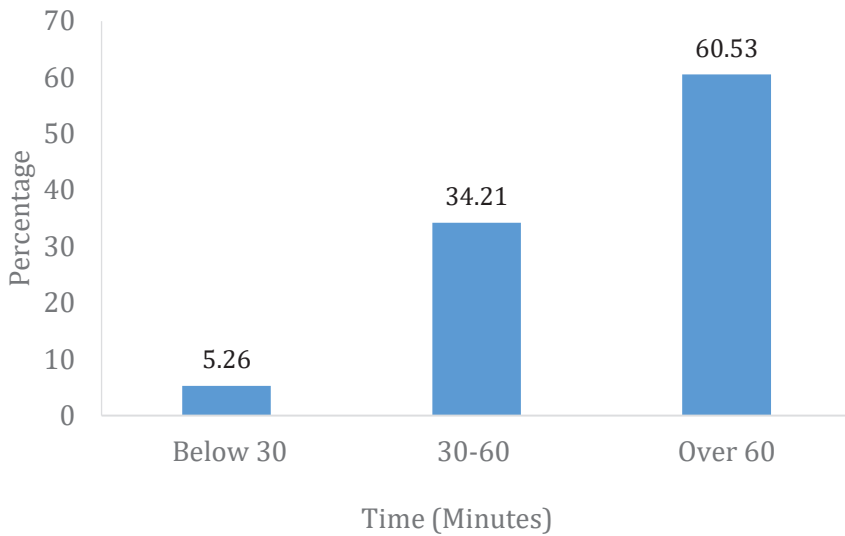


Figure 2: Travel time per day (Source: Survey data, 2020)

Supporting infrastructure facilities of the Agricultural Inspectors to perform their job roles

Importantly, this study focused to analyzing the available supporting factors for the Agricultural Inspectors to perform their job. Accordingly, current knowledge level of the sample towards the technical aspects of their job, adaptation to the new knowledge and their perception towards their satisfaction of the existing knowledge of their job, support of the farmers to perform their job, and the existence of any language barriers to communication with the farmers were measured. This has been done basically to analyze whether they are sufficiently aware of their job roles. Table 1 represents the findings on these aspects.

Table 1: Significance of job performance factors

Indicators	Percentage of respondents	
	Yes	No
Availability of Technical knowledge	21	79
Satisfaction of the current knowledge on their job	95	5
Adoptability of the new knowledge and technology	98	2
Farmers support to perform job well	87	13
Existence of any Language barriers to perform job well	32	68

(Source: Survey data, 2020)

As per Table 1, 79% of the responded sample was already having enough technical knowledge for extension related activities. The remaining 21% do not have enough technical knowledge in extensions activities. Importantly, 95% of Agricultural Inspectors have sufficient knowledge/ skills related to the extension while around 5% do not have sufficient knowledge/ skills related to the extension. Similarly, the majority of

respondents (60.5%) of the sample had high adaptation for the new technology while 39% respondents had moderate adaptation for the new technology and others 1.5 % had low adaptability to new knowledge. In context to communication barriers due to language issues, 68% of the respondents did not have any language barriers in their working area and 32% of respondents have faced language problems during their service duration.

Impacts of motorcycle subsidy on job performance

Furthermore, this study has analyzed the impact of motorcycle subsidy on the job performances of the Agricultural Inspectors using 13 variables and using an average score of each variable. According to research findings, Agricultural Inspectors were able to save their time through less travel time and thus obtained time to improve their job roles through accessing new knowledge.

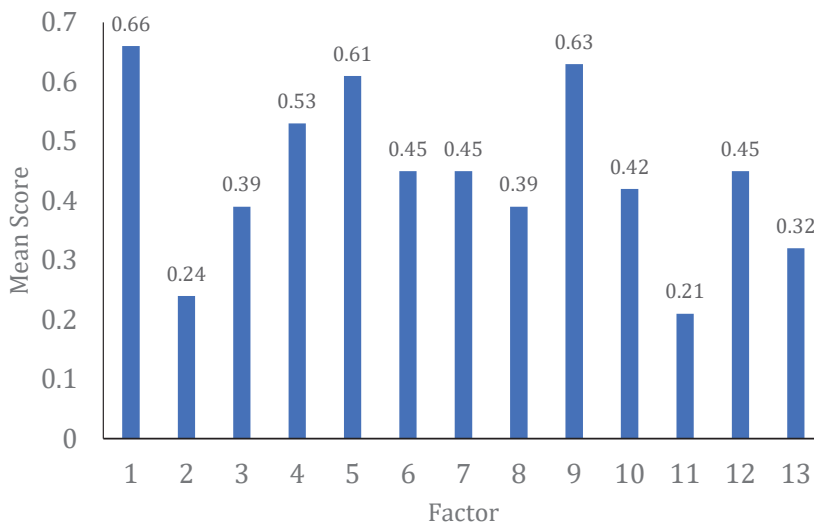


Figure 3: Mean scores of job performance factors. 1 – Improved job role, 2 – Job description not same, 3 – Improved job engagement, 4 – Covered extra area than previous, 5 - Cover few more farmers, 6 - Improved relationships with farmers, 7 - Feel more interesting, 8 - Built-up strong relationships, 9 - Time for knowledge enhancement, 10 - Inadequate transport reduce job performance, 11 – Effect of distance in job performance, 12 - Can perform well in short distance, 13 - Time for non-extension works. (Source: Survey data, 2020)

Table 2: Significance of job performance factors

Job performance factors	Sig. (p)	Test value	Decision
Improved job role	0.001	0.203	Subsidy has significantly impacted on the job improvement
Changing of the job description	0.113	0.002	Subsidy has not significantly impacted on any changes of the job description
Improved job performances	0.068	0.061	Subsidy has not significantly impacted on the improvement of job performances
Greater extent of area can be covered after the subsidy	0.011	0.482	Subsidy has significantly impacted on the extension covering area
Higher number of farmers can be accessed within a day after the subsidy	0.013	0.814	Subsidy has significantly impacted on no. of farmer visit
Improved the relationship with co-workers	0.040	0.600	Subsidy has significantly impacted to relationship improvement with co-workers

Significance at 5% level
(Source: Survey data, 2020)

Table 2: Continued

Job performance factors	Sig. (p)	Test value	Decision
Improved interest towards the job	0.033	0.520	Subsidy has significantly improved job interest
Improved the relationship with farmers	0.037	0.120	Subsidy has significantly help to improve relationship with farmers
Having sufficient time for knowledge dissemination	0.002	0.203	Subsidy has significantly impacted to save time for the knowledge dissemination process
Distance from home to workplace does not affect the job performance	0.315	0.402	Subsidy has significantly impacted to the workplace distance
Increase job performance if the distance between workplace and home is less	0.026	0.821	Subsidy has significantly improved job performances by saving traveling time
Time used for non-extension activities are less after having the subsidy	0.115	0.008	Subsidy has significantly saved time to engage non-extension activities.

Significance at 5% level
(Source: Survey data, 2020)

Table 3: Impact of motorcycle subsidy on the personal wellbeing of the Agricultural Inspectors

Well-being factors	Sig. (p)	Test value	Decision
Improved personal security when travelling	0.227	0.720	Subsidy has not significantly improved the personal security when travelling.
Balanced work-life	0.508	0.400	Subsidy has not significantly impacted on balanced work life.
Saving time for knowledge enhancement	0.267	0.208	Subsidy has not significantly impacted on saving time for knowledge enhancement
Use of leisure time for productive activities	0.372	0.177	Subsidy has not significantly impacted on saving time for leisure activities.
Time saving for social interactions	0.623	0.008	Subsidy has not significantly impacted on saving time for social interactions.
Impact of personal health status	0.010	0.710	Subsidy has significantly impacted personal health status.

Significance at 5% level
(Source: Survey data, 2020)

More importantly, they were able to visit a few more farmers due to less traveling time resulting from subsidy which shows the positive impact of these subsidy programs. Figure 3 shows the mean score of each variable. Furthermore, this study has used an independent sample t-test to analyze the statistically significant impact of motorcycle subsidy schemes on the job performances factors of Agricultural Inspectors.

Impact of motorcycle subsidy on the well-being of the Agricultural Inspectors

Other than the impact of the job performances, motorcycle subsidy scheme might have a similar impact on the well-being of the Agricultural inspectors. Therefore, this study has focused on analyzing the impact of the subsidy scheme on the different dimensions of wellbeing of the Agricultural Inspectors using the mean score of the 6 variables. Based on the research findings, motorcycle subsidy scheme has saved the time of the Agricultural Inspectors for their other activities and enhanced personal security to a greater extent (mean score: 0.21). Moreover, Table 3 shows the results of the independent sample t-test. Accordingly, the subsidy scheme has shown a significant impact only on the personal health status of the Agricultural Inspectors. These results can be justified as the time saved due to convenient travelling mode which has been used for the maintenance of personal health check-ups, exercises, and mental relaxation activities.

CONCLUSIONS

The main objective of the study is to analyze the impact of motorcycle subsidy schemes on the performances and personal wellbeing of the Agricultural Inspectors of the southern province. Accordingly, first, this study has identified the general constraints of extension officers' job performance and their knowledge dissemination. Accordingly, the study has found that the covering area of each Agricultural Inspectors assigned to be covered is about nearly or more than 10 km per day and this has been found to be a major constraint for the Agricultural Inspectors to cover such a large area within a single day. Moreover, the number of farmers to be covered within a day has been identified as a constraint. Even though the standard number of farmers covering per day is five, the majority of Agricultural Inspectors in this study has to visit more than ten farmers per day. Each Agricultural Inspectors has to spend the cost of greater than Rs.100 per day to travel this area which also leads to dissatisfaction

towards their job. This cost has to be covered using their personal budget and the government does not have any plan to pay this traveling cost to Agricultural Inspectors. In summary, Agricultural Inspectors in this study were not satisfied with their traveling time, infrastructure facilities in the working area. Therefore, the government later has decided to allocate this subsidy to minimize the aforementioned issues.

When considering the impact of motorcycle subsidy on the job performance, there was a significant impact on improved the job role, improved job performances, covering a greater land area, accessing a higher number of farmers, improved the relationship with co-workers, improved interest towards the job, improved the relationship with farmers, having sufficient time for knowledge dissemination and Increase job performance if the distance between workplace and home is less are significant. Moreover, the motorcycle subsidy has significantly impacted the personal health of the Agricultural Inspectors.

RECOMMENDATIONS

Although the government is trying to maintain the healthiest working environment for the Agricultural Inspector to improve their working efficiency through this subsidy scheme, data analysis indicates that Agricultural Inspectors were not much benefited and improved through the existing motorcycle subsidy provided by the government. Also, they are expecting more developments of their subsidies and facilities, and therefore, the government should try to maintain a good well-being program to enhance the Agricultural Inspectors' willingness to retain within the extension service. More importantly, the effectiveness of the motorcycle subsidy given by the government on agricultural extension service should be regularly and formally evaluated and decide whether this subsidy program to be continued with new recruiters also.

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DECLARATION OF CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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Biochemical characterization and antibacterial potential of lactic acid bacteria from *Idli* batter and their influences on properties of batter

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Abstract

Idli is one of the flour-based foods fermented by lactic acid bacteria. This study was designed to characterize the lactic acid bacteria isolated from *Idli* batter and to determine their antibacterial activity. Physicochemical changes of batter during fermentation and sensory qualities of the final product (*Idli*) also were determined. Lactic acid bacteria were isolated from *Idli* batter and characterized up to genus level. Agar well diffusion assay was used to determine the antibacterial activity of isolates against foodborne pathogens (*Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus*). Changes of pH, lactic acid bacterial count, titratable acidity of batter during fermentation and sensory properties of the final product were measured. Ten isolates (I-1 to I-10) were isolated from *Idli*

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batter, of which, six were rod and other four were cocci shaped. All ten isolates were Gram positive, non-motile, non-spore formers and catalase activity negative. It was confirmed that all isolates belong to lactic acid bacteria up to genus level. Based on diameter of the inhibition zone, among ten isolates, I-6 was considered as the highest potential bacteriocinogenic isolate. Inhibition zone diameter of isolates ranged from 7.3 ± 1.53 to 16.3 ± 0.58 mm. The pH dropped steadily from 6.28 to 3.72, while titratable acidity increased from 0.24 to 0.92% throughout the fermentation period (0-32 h). With fermentation, the lactic acid bacterial count was increased, and highest lactic acid bacterial count of $9.88 \log_{10}$ cfu/g was observed after 12 h of fermentation. *Idli* prepared from *Idli* batter after 8-12 h of fermentation scored maximum for the sensory quality. From this study it is concluded that all lactic acid bacteria isolates possess antimicrobial activity and physicochemical and sensory qualities of *Idli* batter changed with duration of fermentation.

Keywords: antimicrobial activity, *idli* batter, lactic acid bacteria, physicochemical, sensory quality

INTRODUCTION

Fermented foods have acquired an ever-increasing trend due to their health benefits and fermentation is one of the oldest food preservation methods. Fermented foods are popular nowadays across the world as they are considered as functional foods. The Lactic Acid Bacteria (LAB) are the dominant organisms involved in fermentation of foods and produce acids in optimum amount. The amount of acid production varies with type of food and it changes the sensory quality of food.

LABs are important group of probiotic bacteria. They have become a major focus in food industry as they are improving human health in natural way (Fernandez *et al.*, 2003; Balasubramanian and Viswanathan, 2007). These organisms have been widely reported to exert many beneficial health effects, such as activation of immune system, prevention of cancer cell growth, maintenance of mucosal integrity and presentation of an antagonistic environment for pathogens (Rashmi and Gayathri, 2014).

It is important to isolate and screen the new probiotic LAB strains from fermented foods as they own strong probiotic functionalities. Antimicrobial activity against foodborne pathogens is one of such

probiotic functionalities. They have ability to produce organic acids, exopolysaccharides and proteolytic products and through these by products they show antimicrobial activity against many foodborne pathogens (Abd El Gawad *et al.*, 2010; Ayad *et al.*, 2004; Ayad and Shokery, 2011; Chelliah *et al.*, 2016; El-Soda *et al.*, 2003).

Fermented foods from Asian recipe are non-dairy products, prepared from different raw materials, such as vegetables, grains, fruits, etc. Relatively, rapid growth is initiated by LAB over a wide range of salt concentrations and temperature (25 to 37 °C) in numerous plant-based flour materials. "Dosa", yoghurt rice, "Idli" and hoppers are considered as some of traditional fermented products consumed by vast majority of the people in Sri Lanka and India for their breakfast and dinner (Wickramanayake, 2002).

Idli is a cereal and legume based fermented food. Raw materials used for the preparation of *Idli* batter are rice (*Oryza sativa*) and dehulled black gram (*Phaseolus mungo*). Following fermentation, the *Idli* batter is steamed and formed into round shaped, soft and porous textured product. Some studies have reported that LABs are involved in *Idli* batter fermentation and have probiotic potential. Those strains are *Lactobacillus collinoides* (Patel *et al.*, 2012), *Leuconostoc mesenteroides*, *Streptococcus faecalis*, and *Pediococcus cerevisiae* (Mukherjee *et al.*, 1965) (Balasubramanian and Viswanathan, 2007), *Lactobacillus delbrueckii* (Soni *et al.*, 1986), *Lactococcus lactis* and *Lactobacillus plantarum* (Iyer *et al.*, 2013). The LAB isolates from *Idli* batter also can be used as a starter culture during the fermented (functional) food production (Conter *et al.*, 2005).

Even though, several studies have shown the biochemicals produced by LAB during fermentation of *Idli*, activity of LAB in the gut and their contribution for sensory quality of food, still, isolation and screening of new probiotic strains of LAB from fermented foods is a major research focus. *Idli* batter is fermented by LAB and the LAB isolated from this batter can be used for innovative industrial food applications. Considering the above facts, this study aimed to isolate and characterize the novel strains on LAB from *Idli* batter, determine the antibacterial activity of isolates and identify the acidity changes and sensory quality of *Idli* batter with fermentation.

MATERIALS AND METHODS

Materials

Black gram (*Phaseolus mungo*) and parboiled rice (*Oryza sativa*) were procured from local market in Vavuniya, Sri Lanka. All microbiological media and chemicals were obtained from HiMedia, India. The standard antibiotic discs were procured from Oxoid, UK. The pure cultures of standard food borne pathogens (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica*) were collected from Palmyrah Research Institute, Jaffna, where it was isolated and preserved. The culture was tested again for purity and species characteristics for confirmation.

Preparation of *Idli* batter

Idli batter was prepared by the procedure described by Wickramanayake (2002). The black gram and parboiled rice were soaked in water separately for four to six hours. The seed coat of black gram was removed, mixed in the proportion of 1 to 2, mashed and left overnight at ambient conditions.

Isolation of LAB

Agar microbial growth media, deMan Rogosa and Sharpe (MRS) was used for the isolation of LAB from the batter as reported previously by De Man *et al.* (1960). The pH of the MRS medium was maintained at 6.2. *Idli* batter (1 g) was suspended in 9 mL of 0.9% saline water and subjected to serial dilutions. Diluted mixture (0.1 mL) was pipetted out and spread out on the surface of an MRS medium agar plate. The plates were incubated in the inverted position for 48 h at 37 °C under anaerobic conditions using candle jar technique.

Ten well developed characteristic colonies growing on the MRS medium were picked up carefully and purified by streaking on MRS agar medium plates following four-way streaking technique and subsequently they were purified for 4 to 5 times to get pure cultures. The discrete single purified colonies were picked up and transferred into MRS broth in culture vials and the grown-out cultures were maintained at 4 °C in a refrigerator for further analysis (Pal *et al.*, 2005). To prepare fresh pure cultures for further analysis, the preserved pure cultures were activated in MRS agar by streaking and incubated at 37 °C under anaerobic condition for 48 h before experiments.

Identification and biochemical characterization of pure culture

Gram staining as described by Rangaswami and Bagyaraj (1993) was carried out for morphological examination. Catalase activity (Murry, 1981), motility test (Barrow and Felthman, 1993) and spore forming ability (Barrow and Felthman, 1993) were used for biochemical identification of LAB from *Idli* batter.

Gram staining

The Gram characteristics of LAB were determined for the fresh isolates grown over 48 h using light microscope following staining. The gram-stained slides were observed under oil immersion (10 x 100) of light microscope (Olympus CH20i-India) and the cell morphology of isolated LAB were observed as described by Becking (1974).

Catalase test

A loopful of LAB culture grown over 48 h were transferred into a glass test tube containing 0.5 mL distilled water and mixed thoroughly with 0.5 mL of 3% hydrogen peroxide solution. Subsequently, the effervescence was observed.

Motility test

“Hanging drop method” was used to examine the motility of strains. At the centre of a coverslip, the bacterial culture was placed and each corner of the coverslip a drop of paraffin wax was placed. Over the coverslip a cavity slide was placed, and it was inverted to suspend the bacterial culture in the cavity slide’s central depression. Finally, the motility was observed under high-power dry objective (x40) with reduced illumination of light microscope (Olympus CH20i-India).

Endospore staining

On a microscopic slide, a thin smear of bacterial smear was prepared under aseptic conditions and heat fixed. Then the slide was placed over a steaming water bath and malachite green (primary stain) was applied for 5 min on the slide while covering the slide with blotting paper. Then the slide was removed from the water bath, the blotting paper was removed, and the slide was rinsed with water until water turned clear. Then the slide

was flooded with the counter stain safranin for 20 s and rinsed with water. Subsequently the slide was blot-dried and observed using oil immersion (10x100) of light microscope (Olympus CH20i-India).

Estimation of the growth of LAB count with fermentation

LAB counts were determined with fermentation time by using spread plate method (SLS: 516: Part 1: 1991). The freshly prepared *Idli* batter was allowed to ferment and during fermentation, samples of nearly 25 g were obtained from *Idli* batter for every 4 h interval up to 32 h and LAB counts were determined.

Serial dilutions were prepared up to 10^{-5} . Samples (0.1 mL) were taken from each dilution and poured on a MRS media plate. The sample was spread out evenly over the surface of agar using the sterile glass spreader, while the underneath of petridishes were rotated at the same time. Then, the plates were incubated in an inverted position for 48 h at 37 °C under anaerobic condition by candle jar technique. Three replicates were maintained. After the specified period of incubation, the colonies were counted in each dish containing not more than 300 colonies using the colony counter.

Evaluation of the biochemical changes during fermentation of batter

Idli batter was allowed to ferment, and pH (Sension+ PH 31-Spain) and titratable acidity were determined every 4 h interval up to 32 h of fermentation.

Evaluation of sensory quality of *Idli* with different fermentation time ***Idli***

Idli prepared by fermenting the *Idli* batter for different time durations: 4, 8, 12 and 16 h were subjected for sensory attributes based on a 5-point hedonic scale using a panel of 30 semi-trained panellists.

Assessment of antimicrobial activity of isolated LAB

Antimicrobial activity of isolates was assessed against food borne pathogens, *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus* by measuring the diameters of zone of inhibition (Chopra and Mehra, 2015) using agar well diffusion method.

The sample was centrifuged at 5000 rpm for 15 mins. The resulting cell debris that formed a pellet was discarded giving rise to a cell free supernatant. The pH of the Cell Free Supernatant (CFS) was adjusted to 7 with 1M NaOH (Sharpe *et al.*, 1979). Sterile cotton swabs were dipped into the culture of test microorganisms; *E. coli*, *S. aureus* and *S. enterica* were inoculated by spread plate method over entire surface of Muller Hinton agar plates, which was preset. Agar well (9 mm) was created using sterile cork borer. CFS was poured into the wells and kept into the refrigerator at 4 °C for 2 h. Later, the plates were incubated anaerobically at 37 °C for 24 h. After 1 day of incubation at 37 °C, each plate was examined for zone of inhibition. The diameter of inhibitory zone was measured.

Statistical analysis

Three independent experiments for each isolate were carried out to determine the titratable acidity, pH, LAB count and antibacterial potential. The means of variable and standard deviations were calculated using Microsoft excel 2010. The data were analyzed using one way analysis of variance (ANOVA) using SPSS 16.0. The significant difference was compared using Turkey test.

RESULTS AND DISCUSSION

Ten well developed characteristic presumptive LAB were isolated from *Idli* batter. The characteristic colonies were identified on the surface of MRS agar petri plates. Different type of colonies: punctiform, small, moderate and large size colonies were observed on the surface. All colonies were non-pigmented in other words, creamy to white (Table 1).

Based on Gram staining, all isolates were Gram positive, 6 isolates were rod shaped while other 4 were cocci shaped. In case of endospore test, the isolates without green stained spores were termed as non-spore formers. All cultures were shown negative result thus they were identified as non-spore formers. The motility test has recognized that all isolates shown non-motile behaviour. Catalase test is used to determine if bacteria possess catalase enzyme which can detoxify H₂O₂. The LABs are negative to catalase test as they neither possess peroxidase enzyme nor produce bubbles when mixed with 3% hydrogen peroxide (Murry, 1981). Catalase test for all ten isolates were negative. indicating the absence of catalase enzyme and confirms that the isolates belong to LAB.

Table 1: Morphological and Biochemical characteristics of LAB isolates from *Idli* batter

Isolates	Colony morphology	Gram reaction	Motility	Spore forming ability	Catalase activity	Genus Name
I-1	Circular, Moderate size, White creamy and Smooth	Positive, cocci	Non-motile	Non-spore formers	Negative	<i>Lactococcus</i> spp.
I-2	Circular, Large size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	<i>Lactobacillus</i> spp.
I-3	Circular, Punctiform, White creamy and Smooth	Positive, cocci	Non-motile	Non-spore formers	Negative	<i>Lactococcus</i> spp.
I-4	Irregular, Moderate size, White creamy and Smooth	Positive, cocci	Non-motile	Non-spore formers	Negative	<i>Lactococcus</i> spp.
I-5	Circular, Small size, White creamy and Smooth	Positive, cocci	Non-motile	Non-spore formers	Negative	<i>Lactococcus</i> spp.
I-6	Spindle, Large size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	<i>Lactobacillus</i> spp.
I-7	Irregular, Small size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	<i>Lactobacillus</i> spp.
I-8	Irregular, Large size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	<i>Lactobacillus</i> spp.
I-9	Spindle, Moderate size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	<i>Lactobacillus</i> spp.
I-10	Spindle, Small size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	<i>Lactobacillus</i> spp.

From 10 isolated colonies, 6 isolates were found to be Gram positive, rod shaped, non-motile, non-spore formers and negative to catalase activity, belonging to *Lactobacillus* spp. Other 4 isolates were Gram positive, cocci shape, non-motile, non-spore formers and negative to catalase activity, belonging to *Lactococcus* spp. In summary, according to the above tests, it was confirmed up to the genus level that all strains were LAB and among them 6 were *Lactobacillus* spp.

The antimicrobial activity as measured by the zone of inhibition is given in Table 2. Among ten isolates, isolate I-6 was considered as the highest potential bacteriocinogenic isolate against all test organisms. Smaller zone of inhibition was noted by all isolates against *E.coli*. In case antagonism activity of isolates, isolates I-2, I-3, I-5, I-6, I-7 and I-10 were shown highest antagonism activity against *S. enterica*, than other indicator organisms.

Table 2: Antimicrobial activity of LAB isolates obtained from *Idli* batter

Isolates	<i>Salmonella enterica</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
1	12.3±0.58 ^{bcd}	9.3±1.53 ^{ab}	12.7±0.57 ^{ab}
2	16.3±0.58 ^a	8.3±1.53 ^{ab}	9.6±1.15 ^b
3	14.7±1.15 ^{ab}	8.3±1.53 ^{ab}	10.7±0.58 ^{ab}
4	9.7±0.58 ^{de}	8.7±1.15 ^{ab}	11.3±0.58 ^{ab}
5	13.3±1.53 ^{bc}	7.3±1.53 ^b	12.7±1.15 ^{ab}
6	16.3±0.58 ^a	11.7±1.15 ^a	13.3±1.53 ^a
7	14.3±0.58 ^{abc}	11.7±0.58 ^a	12.7±1.15 ^{ab}
8	9.3±1.53 ^e	10.3±1.53 ^{ab}	12.7±0.58 ^{ab}
9	10.3±1.53 ^{de}	11.3±0.58 ^a	12.3±1.53 ^{ab}
10	11.7±0.58 ^{cde}	10.7±0.58 ^{ab}	11.7±1.15 ^{ab}

Each value in the table was represented as mean ± SD (n = 3). Values in the same column followed by a different letter (a-e) are significantly different (p < 0.05).

The isolates I-1, I-4, I-8, I-9, and I-10 was shown higher zone of inhibition against *S. aureus*, than other indicator organisms. The overall zone of inhibition diameter of isolates were fallen within the range from 7.3 ± 1.53 to 16.3 ± 0.58 mm. Tejero-Sarinena *et al.* (2012) has stated that production of antimicrobial substances such as hydrogen peroxides, acetic acid, lactic acid and bacteriocins are the reason for showing antimicrobial properties by probiotics.

The main physiochemical characteristic of *Idli* batter fermentation is lactic acid formation. It can be noted by changes in the pH and titratable acidity. Table 3 shows the changes of pH, titratable acidity, and LAB count during fermentation of *Idli* batter. The measurements were taken for every 4 h time interval up to 32 h of fermentation. During fermentation, the titratable acidity was increased slowly from 0.24 to 0.32 % at the 8 h of fermentation *Idli* and sudden increment has been noted at 8 - 12 h of fermentation from 0.32 % to 0.40 %. After 12 h, the titratable acidity remained steady up to 24 h and the titratable acidity unchanged in both 24 and 28 h of fermentation that was 0.52 %. At the period of 28 - 32 h the titratable acidity increased drastically from 0.52 % to 0.92 %. While titratable acidity increased from 0.24 to 0.92%, the pH dropped steadily from 6.28 to 3.72. Even though the fermentation temperature varied between 25 and 37 °C, the same observation has been recorded in several studies (Ghosh and Chattopadhyay, 2012; Rajalakshmi and Vanaja, 1967; Soni and Sandhu, 1989; Soni and Sandhu, 1990; Thakkar *et al.*, 2015).

The increasing acidity in *Idli* batter mainly depends on *Streptococcus faecalis* as it produces lactic acid and reduces the pH, increases CO₂ concentration in the batter, which leavens batter property (Mukherjee *et al.*, 1965). With the growth of LAB, the pH of batter decreases. The reduction of batter pH helps the activity of yeast as the optimum pH for yeast ranges from 4.4 to 4.5 (Soni and Sadhu, 1990).

The LAB count increased from 7.55 to 9.88 log₁₀cfu/g during fermentation. High LAB count was observed after 12 h of fermentation *Idli* where the pH level was noted as 4.64. The count reduced with the increment of acidity. After 12 h of fermentation the LAB count dropped drastically from 9.88 to 5.91 log₁₀cfu/g during 32 h of fermentation. It may be due to increment of acidity which may not be favourable to LAB population.

The sensory quality of *Idli* prepared from the batter with different fermentation durations was analysed (Figure 1). The colour, appearance,

taste, texture, mouth feel and the overall acceptability were taken into the analysis. The *Idli* prepared following 8 and 12 h of fermentation showed maximum desirable sensory quality where pH and acidity were shown within the range of 4.64 - 5.21 and 0.32 - 0.40%, respectively.

Table 3: Changes of pH, titratable acidity and LAB count with fermentation of *Idli* batter at 4 h time intervals from 0-32 h

Fermentation time (h)	pH	LAB (cfu/g)	Titratable acidity (%)
0	6.28±0.02 ^a	7.55±0.11 ^d	0.24±0.02 ^e
4	5.86±0.01 ^b	8.11±0.1 ^c	0.28±0.01 ^{de}
8	5.21±0.06 ^c	8.89±0.02 ^b	0.32±0.07 ^{cde}
12	4.64±0.04 ^d	9.88±0.05 ^a	0.40±0.06 ^{bcd}
16	4.58±0.04 ^d	8.74±0.09 ^b	0.44±0.02 ^{bc}
20	4.39±0.07 ^e	7.05±0.07 ^{de}	0.48±0.05 ^b
24	3.88±0.03 ^f	6.84±0.26 ^e	0.52±0.11 ^b
28	3.78±0.08 ^{fg}	6.97±0.15 ^e	0.52±0.04 ^b
32	3.72±0.06 ^g	5.91±0.46 ^f	0.92±0.01 ^a

Each value in the table was represented as mean ± SD (n = 3). Values in the same column followed by a different letter (a-g) are significantly different (p < 0.05).

Idli prepared with the batter fermented for 4 and 16 h scored less for the overall acceptability. It may be due to the characteristic flavour and taste that may develop with prolonged fermentation. Four hours of fermentation might not be adequate to develop the desirable sensory attributes. Following 16 h of fermentation, higher acidity the sourness may be increased, and the activity of microorganisms may cause undesirable texture of *Idli*. It may be the reason for the lower scores in overall acceptability (Figure 1). The maximum score of sensory quality was obtained at the LAB count was noted high in number in the batter which was at 8 h and 12 h of fermentation. Therefore, it indicates that

LAB contribute to the textural and over all acceptability improvement of *Idli* batter. The same results were stated by different authors (Lee, 2001; Thakkar et al., 2015). They reported that the taste of *Idli* prepared from the *Idli* batter when the pH and acidity of *Idli* batter within the range of 4.0 - 4.5 and 0.5 - 0.6% at the time of fermentation has scored maximum.

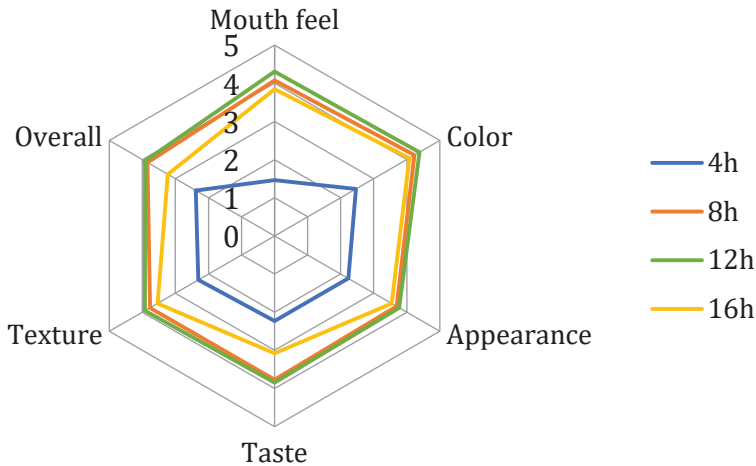


Figure 1: Web diagram representing sensory quality of *Idli* prepared using different fermentation durations

CONCLUSIONS

Lactobacilli spp are the predominant LAB microbial group involved in natural *Idli* batter fermentation which have antimicrobial activity against foodborne pathogens. It changes the biochemical characteristics of the *Idli* batter, subsequently it improves the texture and organoleptic characteristics of final steamed product. In future, species level identification of isolated LABs from *Idli* batter, assessment of exopolysaccharide production ability of selected isolates, amount of bacteriocin production to control foodborne / spoilage bacteria and bacteriocin chemical characterization studies can be carried out to bring this study forward and more useful.

DECLARATION OF CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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Effect of gypsum application on yield performance of groundnut (*Arachis hypogea* L.) Varieties in Kilinochchi district, Sri Lanka

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Abstract

Groundnut (*Arachis hypogea* L.) is an important oil crop grown in Sri Lanka. Application of fertilizer is substantially contributing to the yield increment; however improper management of fertilizer is the specific drawback in quality and quantity in groundnut. Therefore, the application of balanced fertilizer and minerals play an important role in the cultivation of groundnut. An experiment was conducted at the Department of Agronomy, Faculty of Agriculture, Kilinochchi to assess the effect of different rates of gypsum application on yield performance of groundnut varieties from January to May 2019. Two-factor factorial experiment was conducted in Randomized Complete Block Design (RCBD) with three replications. Four different rates of gypsum application such as 0 kg/ha (T_1 - Control), 75 kg/ha (T_2), 125 kg/ha (T_3), and 175 kg/ha (T_4) were used as the first factor and five groundnut varieties, namely Tissa

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(V₁), Lanka Jumbo (V₂), Tikiri (V₃), Indi (V₄), and ANK G1 (V₅) were used as the second factor. All the agronomic practices were done according to the recommendations of the Department of Agriculture. The yield parameters were recorded, and the shelling percentage was calculated. Data were analyzed using SAS 9.1 package to perform ANOVA. The best treatment was identified through the means separation using Duncan's Multiple Range Test (DMRT) at p = 0.05. The yield parameters of fresh and dry weight of pods / plant, hundred pods and seeds weight, number of mature and immature pods and total yield were higher in gypsum applied treatments rather than control and the highest in T₄ (175 kg/ha gypsum) treatment. All the yield parameters were significantly different in Lanka Jumbo and ANK G1 from other varieties. However, the number of mature pods and shelling percentage were similar among the varieties. The highest shelling percentage (96 %) was recorded in Lanka Jumbo under 175 kg/ha gypsum application (T₄). The highest yield parameters were recorded in Lanka Jumbo variety, whereas the lowest was in ANK G1. There was no interaction effect among gypsum application and varieties in the yield parameters. From this study, it can be concluded that the application of 175 kg/ha gypsum (T₄) to the variety of Lanka Jumbo (V₂) can be selected as suitable treatment combination to obtain the substantial yield from groundnut in *Yala* season.

Keywords: groundnut, gypsum, shelling percentage, varieties, yield parameters

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) belongs to the family Fabaceae. It is also called peanut, earthnut, monkey nut, pinda, goober, and manila nut (Beghin *et al.*, 2003). It contains 48 - 50 % of oil, 26 - 28 % of protein, and 11 - 27 % of carbohydrates, minerals, and vitamins (Mukhtar, 2009). Groundnut is having several economic importance like extraction of edible oil, eaten as roasted nut, used to prepare peanut milk, butter, snacks, and confectionaries. Oil of groundnut is used in the industries to produce soap, cosmetic cream, plasters, and oil. After oil extraction, the cake is used as a protein supplement in animal feed. Being a legume crop, groundnut enriches soil nutritional status by fixing nitrogen without draining the nonrenewable energies and without disturbing the agro-ecological balance (Reddy and Kaul, 1986), and thereby increasing the productivity of other crops in the cropping systems.

Groundnut is grown on nearly 23.95 million ha worldwide with a total production of 36.45 million tons and an average yield of 1520 kg ha⁻¹ (FAOSTAT, 2019). In dry and intermediate zones of Sri Lanka, it can be grown as a rainfed crop in highlands during *Maha* season and irrigated crop in paddy lands during *Yala* season. Groundnut is grown mainly in Moneragala, Kurunegala, Ampara, Badulla, Puttalam, and Rathnapura districts of Sri Lanka. It was cultivated to an extent of 11,609 ha with a total production of 21,953 t and an average yield of 1890 kg ha⁻¹ in Sri Lanka (DOA, 2019). In Northern Province of Sri Lanka, groundnut is cultivated in 3,914 hectares of land and its production was 6,305 t (Vavuniya 807 hectares, Mullaitivu 2648 hectares, Kilinochchi 154 hectares, Mannar 170 hectares, and Jaffna 135 hectares) (DOA, 2018 - 2019). Eight varieties of groundnut were released by the Department of Agriculture (DOA) such as Red Spanish, Number 45, Tissa, Walawe, Indi, Tikiri, ANK G1, and Lanka Jumbo and out of these at present, Red Spanish and number 45 are not cultivated in large extent (www.agridept.ac.lk).

Groundnut is grown in well-drained sandy loam or clay loam soil. Deep well-drained soils with a pH of 6.5–7.0 and high fertility are ideal for groundnut cultivation. Balanced use of fertilizer is said to play an important role in sustainable crop production (Afridi *et al.*, 2002). In addition to primary nutrients N, P, K, calcium, and sulfur also plays an important role in enhancing the production and productivity of groundnut. Sulfur is very crucial for the formation of sulfur-containing amino acids and oil synthesis as well as it is also improving both the yield and quality of crops (Patel and Patel 1994). Calcium nutrition is also considered a yield-limiting factor for groundnut production. Calcium absorbed by the roots is not translocated to the developing pod whereas calcium required for pod formation is absorbed directly from the soil solution. Calcium and sulfur as main nutrients also act significant position in raising yield and efficiency of groundnut. Sulfur is incredibly vital for the creation of amino acids with sulfur and oil production, and it also enhances yield as well as quality (Kalamkar, 2006).

The application of calcium (CaCO₃) is important for proper kernel development in groundnut (DOA, 2006). Calcium carbonate can be used as a calcium source but compared to Gypsum; it is slow releasing of nutrients due to less solubility. Therefore, gypsum (CaSO₄·2H₂O) can be used at the flowering stage to ensure the adequate availability of Ca in the fruiting zone to enhance the pod development. Chapman *et al.* (1993) reported that the less amount of soluble calcium in the pegging zone causes low

peg formation. The researchers found that the groundnut pegs and pods treated with gypsum had significantly less pod rot than the untreated.

There is no extensive study was done regarding the rate of gypsum application and its impact on the yield of groundnut especially in Northern region of Sri Lanka. The use of gypsum may be considered as an important factor for increasing groundnut yield in Northern area. With this view, the present study was undertaken to evaluate the effect of different rates of gypsum application on yield performance of different groundnut varieties in Kilinochchi District of Sri Lanka. The main objective of this study was to assess the impact of varying rates of gypsum application in yield responses of selected groundnut varieties. Sub-objective of the studies was to evaluate the number of pods and shelling percentage under the different rates of gypsum application with different varieties of groundnut.

MATERIALS AND METHODS

A field experiment was carried out at the Faculty of Agriculture, Ariviyal Nagar, Kilinochchi (belongs to the agro-ecological region of DL₃) from January to May 2019. The experimental site soil is well drained, clay loam in texture. The area receives mean annual rainfall ranges from 1040 mm to 1560 mm. The daily mean minimum and maximum temperature is 28 °C and 33 °C, respectively. Two-factor factorial experiment was carried out in Randomized Complete Block Design (RCBD) with three replications. Different rates of gypsum such as 0 kg/ha (T₁, Control), 75 kg /ha (T₂), 125 kg /ha (T₃), and 175kg /ha (T₄) were used as the first factor, and five groundnut varieties *viz.* Tissa (V₁), Lanka Jumbo (V₂), Tikiri (V₃), Indi (V₄), and ANKG1 (V₅) were used as the second factor. Certified seeds were collected from the district agriculture research and training Centre, Kilinochchi. Unshelled seeds were mixed with captan fungicide (4 g/kg) and kept for 2 - 3 hours. Planting was done in the recommended spacing of 45 cm × 15 cm with the rate of one seed per hill. In each plot, 24 seeds were planted. Gap filling was done by replanting the groundnut plants which were produced in the small cups simultaneously during field planting and the same plant population was maintained in the field for each treatment. All other management practices were carried out according to the recommendations of the Department of Agriculture (<http://www.agridept.gov.lk/index.php/en/crop-recommendations/988>). The earthing-up was done 37 days after planting (Thilini *et al.*, 2018) at the height of 5 cm from the collar region (Ragulan *et al.*, 2016). At the time of earthing-up, the gypsum was applied according to the treatments.

Groundnut varieties were harvested at different periods when those varieties reached maturity by vein yellowing and leaves start to shed. Tissa and ANKG1 varieties were harvested 95 days after planting and Lanka Jumbo, Indi, Tikiri Varieties were harvested at 110 days after planting. After harvesting, the pods were separated from the plants and allowed to sundry for 5 days until the pods are dried.

The yield parameters such as fresh weight of pods per plant (g), dry weight of pods per plant (g), number of mature pods per plant, number of immature pods per plant, 100 pods weight (g), 100 seed weight (g) and the total yield were recorded and shelling percentage (%) was calculated by five randomly selected plants from each plot.

The shelling percentage was calculated using the following equation (Ouedraogo *et al.*, 2012)

$$\text{Shelling percentage (\%)} = \frac{\text{Dry Kernal weight}}{\text{Dry pod weight}} \times 100$$

To find the significant difference between treatments ANOVA was performed by using the statistical package SAS (9.1) and mean separation was done by using Duncan's Multiple Range Test at p value of 0.05.

RESULTS AND DISCUSSION

Fresh pods weight /plant (g)

There were significant differences with the treatments in all varieties at $p=0.05$ (Figure 1). There was no interaction effect between varieties and the rate of gypsum application. The fresh pods' weight /plant was high in gypsum application treatment compared to zero level application (T_1 - Control). The highest fresh pods weight/ plant was observed in the T_4 (175 kg/ha gypsum application) treatment. There was a significant difference in Lanka Jumbo and ANK G1 varieties and other varieties showed a non-significant effect in fresh pods weight/plant (Figure 2). The highest fresh pods weight /plant (136.75 g) was recorded in Lanka Jumbo under T_4 and the lowest (78 g) in ANK G1 under T_1 treatment.

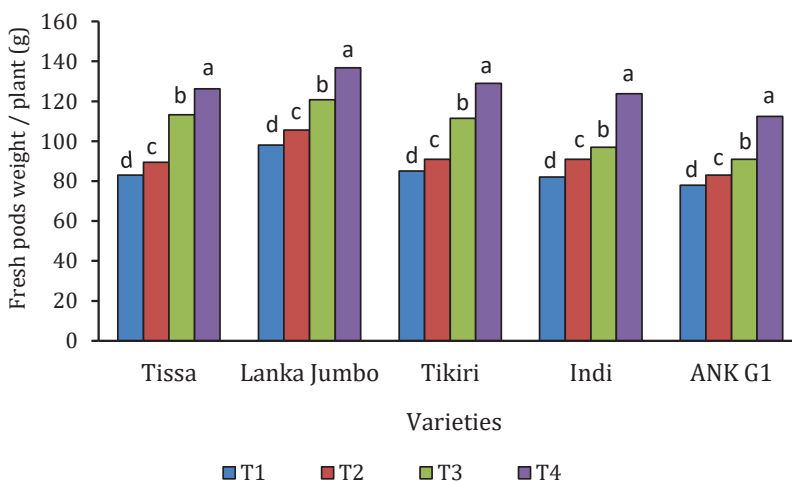


Figure 1: Fresh pods weight /plant with different varieties of groundnut. Means with the same letter within a given variety are not significantly different at p=0.05.

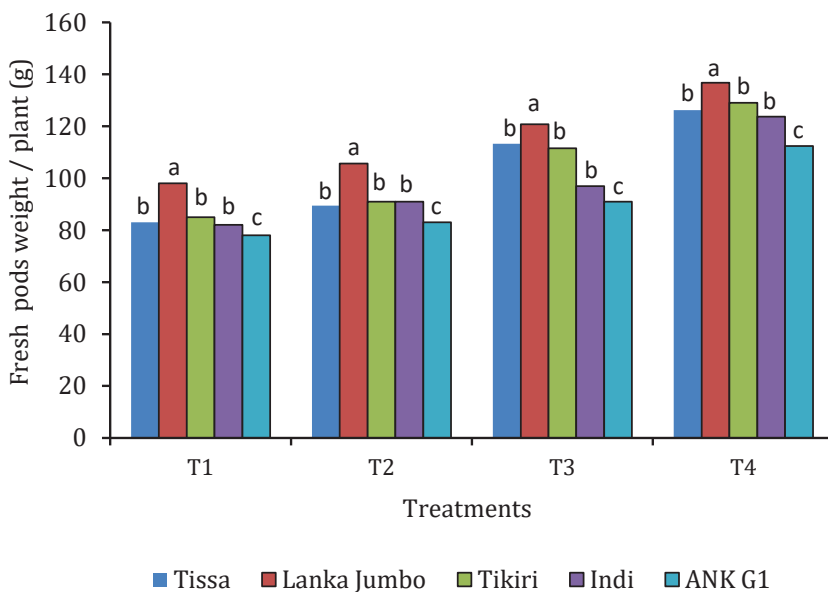


Figure 2: Fresh pods weight /plant with different gypsum treatments. Means with the same letter within a given treatment are not significantly different at p=0.05.

Dry pod weight /plant

Dry pod weight per plant showed the same trend of fresh pods weight per plant. The dry pod weight/plant was high in gypsum application treatments compared to zero level application. The highest dry pods weight/plant was observed in the T₄ (175 kg/ha gypsum application) and the lowest in T₁ (Control). The highest dry pods weight /plant (106.25 g) was recorded in Lanka Jumbo under T₄ and the lowest (67 g) in ANKG1 under T₁ treatment.

Hundred pod dry weight (g)

The hundred pod weight significantly differed among treatments except for T₂ and T₃, but all gypsum applied treatments obtained higher weight than the zero application (T₁). There was no interaction effect between varieties and treatments (Figure 3) There was a significant difference between Lanka Jumbo and ANK G1 (Figure 4). The highest 100 pods dry weight (176 g) was recorded in Lanka Jumbo under T₄ treatment and the lowest (135.36 g) in ANK G1 under T₁ treatment.

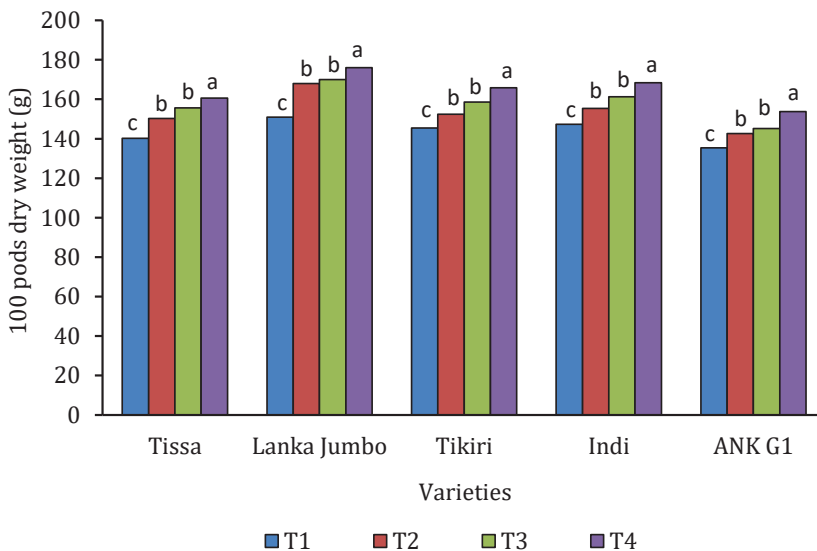


Figure 3: Hundred pods dry weight with different varieties of groundnut. Means with the same letter within a given variety are not significantly different at p=0.05.

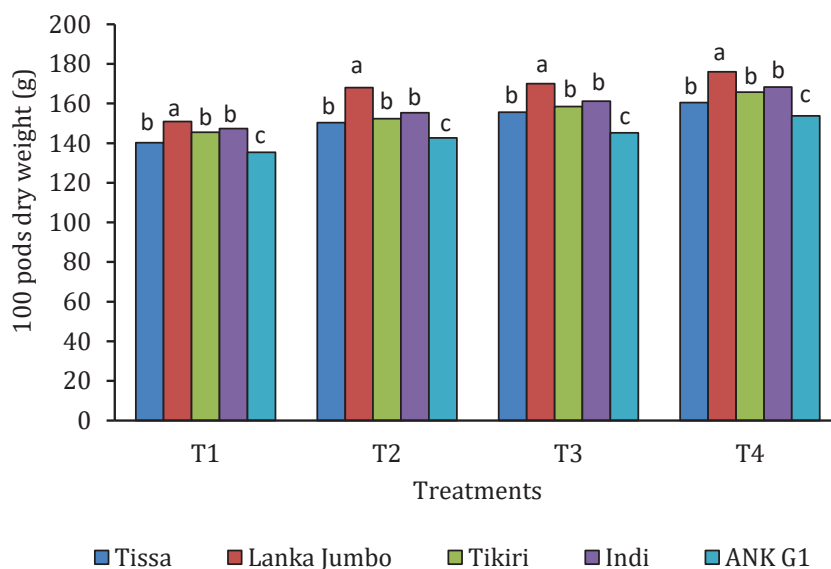


Figure 4: Hundred pods dry weight with different gypsum treatments. Means with the same letter within a given treatment are not significantly different at $p=0.05$.

Hundred seeds dry weight (g)

Similar to hundred pod dry weight, 100 seed dry weight also showed the same trend. The highest 100 seed weight of 95.33 g was observed in Lanka Jumbo under the T_4 treatment and the lowest was observed in the ANKG1 variety under T_1 treatment. The weight of the seed depends on the genetic characters as well as the rate of gypsum application.

Mature pods per plant

The number of mature pods per plant was significantly varied with the rate of gypsum application except for T_3 and T_2 (Figure 5). There was no interaction effect among gypsum application and varieties. Matured pod number was significantly highest in T_4 and lowest in T_1 . There was no significant difference between varieties in the same treatment (Figure 6). The highest mature number of pods of 57 was observed in Lanka Jumbo variety under T_4 and the lowest number of 37 was observed in ANKG1 variety under T_1 . This variation may also be due genetic characteristics of these varieties (Naeem et al., 2015).

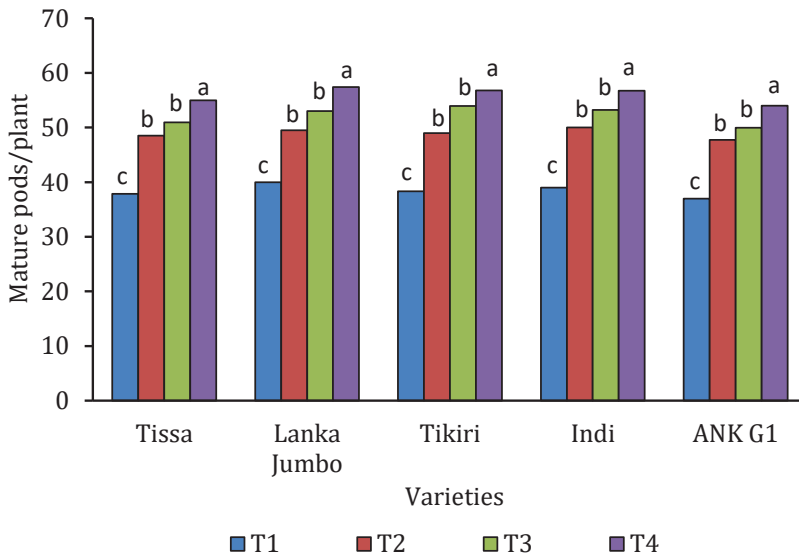


Figure 5: Mature pods /plant with different groundnut varieties. Means with the same letter within a given variety are not significantly different at p=0.05.

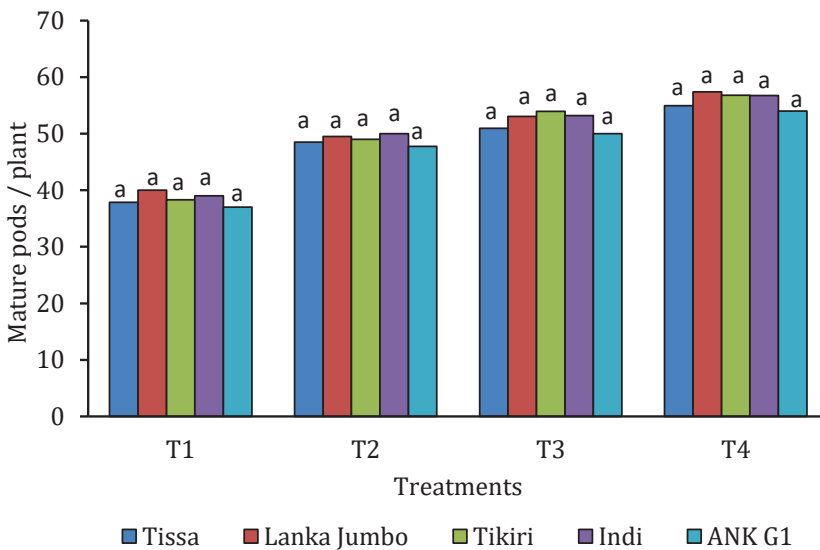


Figure 6: Mature pods /plants with different gypsum treatments. Means with the same letter within a given treatment are not significantly different at p=0.05.

Immature pods /plants

The number of immature pods per plant was significantly differed with rate of gypsum application (Figure 7) in all tested varieties of groundnut. There was no interaction effect among treatments and varieties. The immature pod number was significantly highest in T₁ and lowest in T₄ in all varieties. It may be the effect of gypsum application. There was a significant difference between varieties in the same treatment (Figure 8). The highest immature number of pods of 22 was observed in ANK G1 variety under T₁ (0 level) and the lowest number of 8 was observed in Lanka Jumbo variety under T₄.

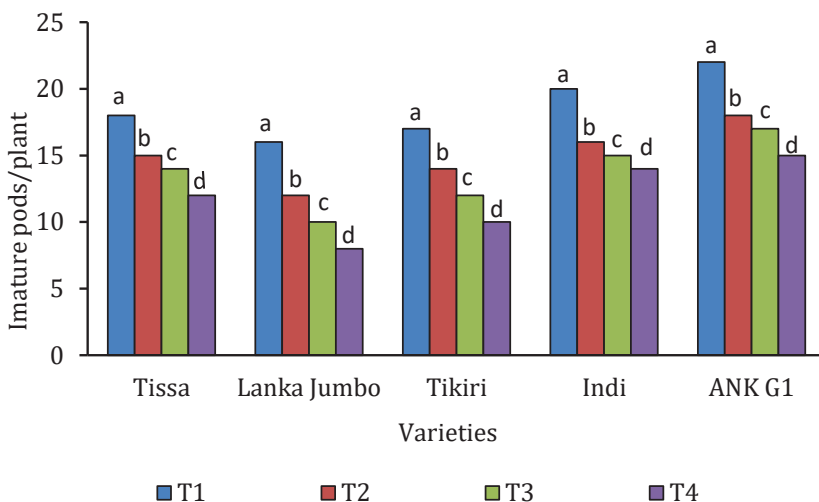


Figure 7: Immature pods per plant with groundnut varieties. Means with the same letter within a given variety are not significantly different at p=0.05.

Shelling percentage

There was a significant difference among treatments in all varieties in shelling percentage (Figure 9). There was no interaction effect between treatments and varieties. The highest shelling percentage was observed in T₄ treatment in each variety. The highest shelling percentage was observed in a variety of Lanka Jumbo compared to other varieties (Figure 10). The highest shelling percentage (96 %) was observed in the Lanka Jumbo variety under the T₄ treatment. A higher shelling percent indicates

less seed case (pod) weight and more seed weight (Jeyaramraja and Woldesenbet, 2014). Ouedraogo *et al.* (2012) indicated that the sandy soil

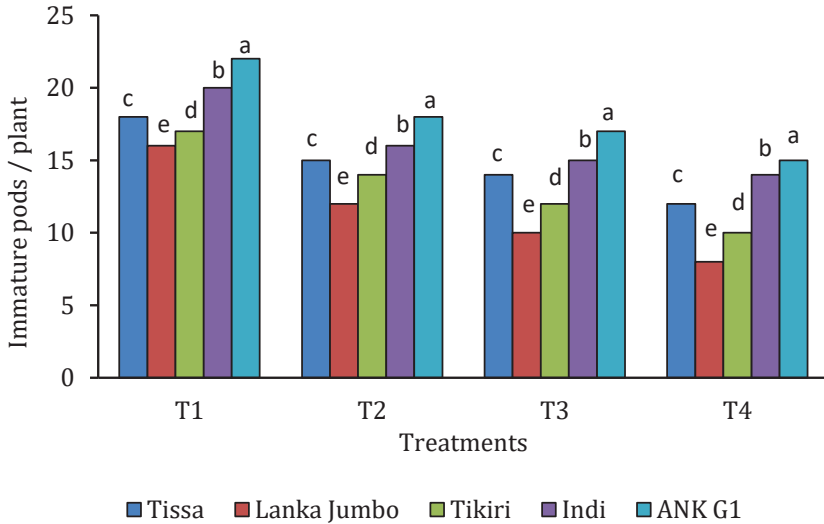


Figure 8: Immature pods per plant with treatments. Means with the same letter within a given treatment are not significantly different at $p=0.05$.

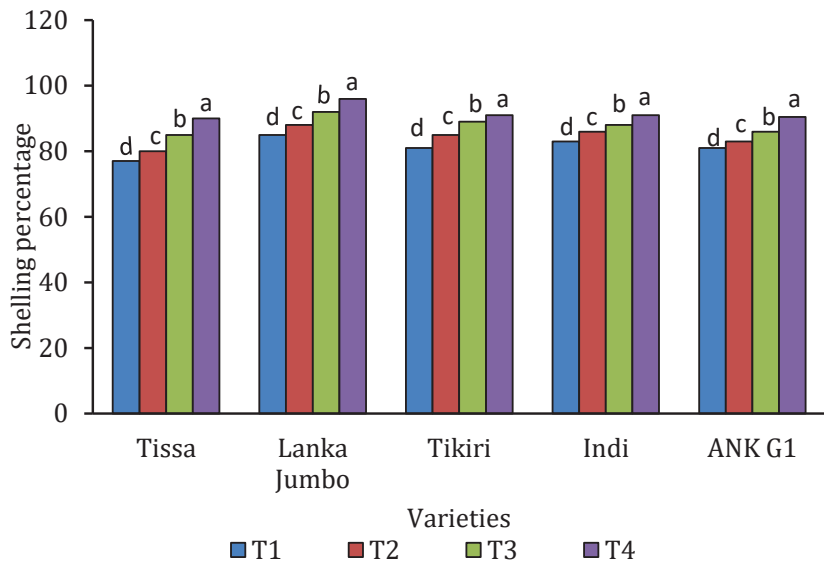


Figure 9: shelling percentage with groundnut varieties. Means with the same letter within a given variety are not significantly different at $p=0.05$.

structure, temperature, and rainfall allowed the expression of the genetic potential of the landraces that had a high rate of pod filling.

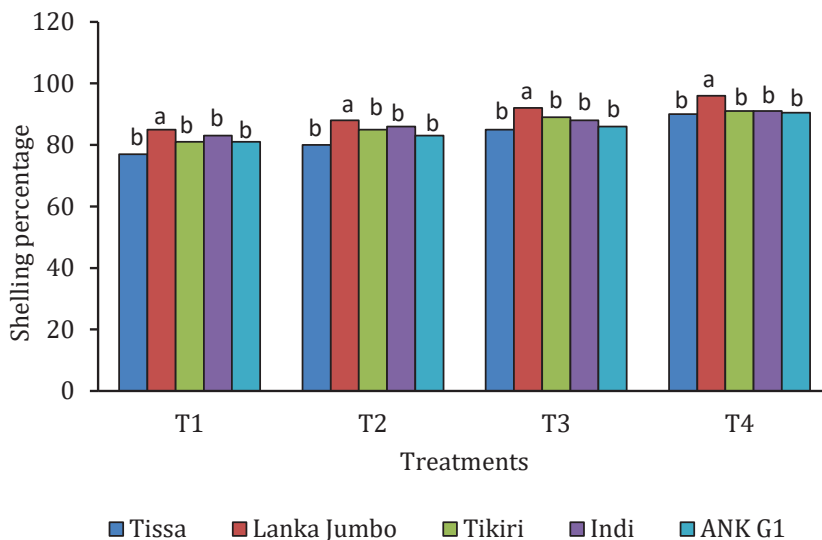


Figure 10: Shelling percentage with treatments. Means with the same letter within a given treatment are not significantly different at $p=0.05$.

Yield of groundnut

The average total groundnut yield (t /ha) was significantly different among the treatments in each variety (Figure 11). There was no interaction effect between varieties and treatments. The highest yield was observed in T_4 treatment and the lowest in T_1 treatment in all varieties. There was a significant difference between varieties except for Tissa, Tikiri, and Indi in the same treatment (Figure 12). The highest groundnut yield (4.5 t /ha) was observed in the Lanka Jumbo groundnut variety (V_2) under the T_4 when compared with the other treatments. The lowest yield of 3.2 t /ha was observed in the ANK G1 (V_5) under the T_1 . The yield was significantly higher in the Lanka Jumbo variety than other varieties. Some research studies demonstrated that groundnut yield increased with applying of fertilizer including sulfur and calcium such as single superphosphate, elemental sulfur, gypsum, and ammonium sulfate in the alkali soils (Maccio et al., 2002; Murata, 2003; Sumner, 1995). Wiatrak et al. (2006) indicated that gypsum application may help to increase peanut yields in years with

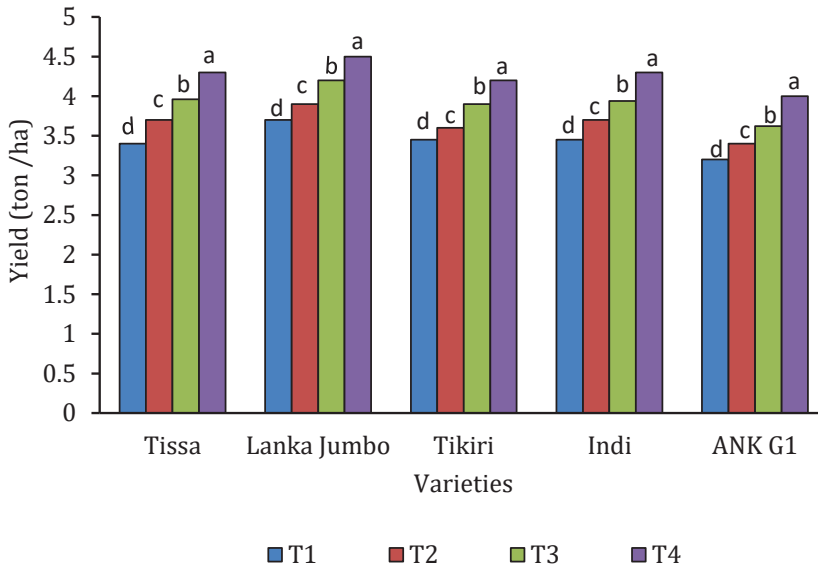


Figure 11: Yield (ton /ha) with groundnut varieties. Means with the same letter within a given variety are not significantly different at $p=0.05$.

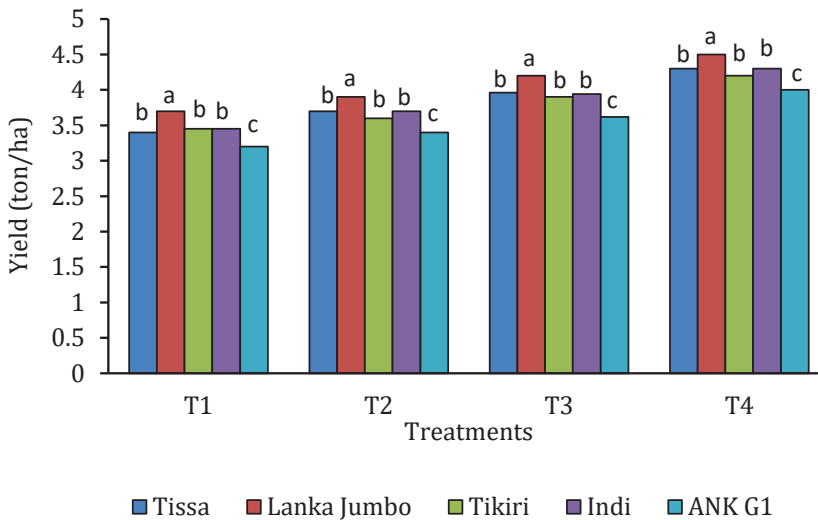


Figure 12: Yield (ton /ha) with treatments. Means with the same letter within a given treatment are not significantly different at $p=0.05$.

high potential yield by increasing Ca availability in the fruiting zone. They reported that peanut yields were higher with gypsum application compared to the treatment without gypsum application.

CONCLUSIONS

The rate of gypsum application of each variety of groundnut significantly influenced the yield parameters. Among the gypsum application, 175 kg/ha showed the highest yield than the other rates of application. Among the varieties, Lanka Jumbo variety gave the highest yield. The highest shelling percentage was obtained in Lanka Jumbo variety under 175 kg/ha gypsum application (T₄). Therefore, Lanka Jumbo variety and 175 kg/ha gypsum application combination can be recommended to Kilinochchi to obtain a high yield in Groundnut.

SUGGESTIONS

This experiment should be repeated during *Maha* season for evaluating the performance of groundnut varieties under different climatic conditions. Research should be carried out with other rates of gypsum application and groundnut varieties.

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Acknowledgements

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...fully hydrogenated fats are blended with liquid oils as the feed stocks of interesterification (Zhang *et al.*, 2000).

.... with low or zero *trans* fatty acids and the results were promising (Abdhu, 2005; Goli *et al.*, 2008; Zhang *et al.*, 2001).

.....*trans*-free margarine formulations and most widely used enzyme for the interesterification is Lipozyme TL IM (Ferreira-Dias, 2013).

.....fatty acids can be obtained by enzymatic interesterification (Huang and Akoh, 1994).

This result was later contradicted by Becker and Seligman (1996).

Reference list

Journal articles

1. Single author

Gil, A. 2002. Polyunsaturated fatty acids and inflammatory diseases. *Biomedicine and Pharmacotherapy*, 56:388–396.

2. Two authors

Huang, K.H. and Akoh, C.C. 1994. Lipase-catalyzed incorporation of n-3 polyunsaturated fatty acids into vegetable oils. *Journal of the American Oil Chemists' Society*, 71:1277–1280.

3. More than two authors

Zhang, H., Xu, X., Mu, H., Nilsson, J., Adler-Nissen, J. and Høy, C.E. 2000. Lipozyme IM-catalyzed interesterification for the production of margarine fats in a 1 kg scale stirred tank reactor. *European Journal of Lipid Science and Technology*, 102:411–418. [https://doi.org/10.1002/1438-9312\(200006\)102:6<411::AID-EJLT411>3.0.CO;2-T](https://doi.org/10.1002/1438-9312(200006)102:6<411::AID-EJLT411>3.0.CO;2-T)

Books and other monographs

4. Chapter in book

Gordon, M.H. 2001. The development of oxidative rancidity. In: Pokorny, J., Yanishlieva, N. and Gordon, M. (eds) *Antioxidants in Food – Practical Applications*. CRC Press, Washington, pp 7-225.

5. Web references

The full URL should be given with the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Example,

WHO. 2015. Eliminating *trans* fats in Europe: A policy brief. http://www.euro.who.int/__data/assets/pdf_file/0010/288442/Eliminating-trans-fats-in-Europe-A-policy-brief.pdf. Accessed January 30 2021

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