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Editor's Note

I am very glad to be an Editor-in-Chief for the Volume 8 of Journal of Dry Zone Agriculture (JDZA) which is published by the Faculty of Agriculture, University of Jaffna, Sri Lanka. The JDZA is set with a keystone of publishing high quality, impactful research findings that address various agricultural and environmental problems with possible scientific solutions. The Volume 8 (Issue I) of the JDZA, this time, comprises of 6 high quality research papers from various fields: Crop science, Soil science, Economics, Environmental sciences and Food technology. The double-blind review process ensured the high quality of the publications published in this Journal. The JDZA has just been connected successfully to the Sri Lankan Journals Online (SLJOL) platform for the wider display to readers all over the world. Moreover, online submission system of the JDZA is now open throughout the year to accept impactful scientific research papers. I, therefore, welcome authors of scientific fields relevant to the scope of the journal to submit their high quality scholarly works to the JDZA for reaching wider scientific display.

Dr.N.Kannan

Editor-in-Chief/JDZA

Content

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Seed Germination and Early Seedling Growth of Different Vegetable Species Treated by Organic Liquid Preparations of *Dasagavya* and *Beejamrita*

K. M. C. Fernando* and E. M. U. I. Ekanayake

Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka

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Abstract

Seed germination and seedling growth are the most crucial stages of the plant life that exert a pivotal influence on production and economic yield. The present study was conducted to examine the effect of *Dasagavya* and, two organic liquid preparations on seed germination and early seedling growth of okra (*Abelmoschus esculentus*) var. MI5, winged bean (*Psophocarpus tetragonolobus*) var. Green, Chinese spinach (*Amaranthus* spp) var. Red, cooking melon (*Cucumis melo*) var. mal, ridge gourd (*Luffa acutangula*) var. local, cabbage (*Brassica oleracea*) var. jumbo, brinjal (*Solanum melongena*) var. *leenairi* and tomato (*Solanum lycopersicum*) var. local. The experiment consisted of eight sub-experiments, was set up as a completely randomized design with four replicates using germination trays and black color pots. *Dasagavya* and *Beejamrita* were used as organic seed treatments and pure water as the control treatment.

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*Corresponding author
Postal address: Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka.
Email: menaka@crop.ruh.ac.lk

The highest final seed germination, Germination Index (GI), Mean Daily Germination (MDG) and the lowest Mean Germination Time (MGT) observed in *Dasagavya* and *Beejamrita* treated seeds compared to the control in eight vegetable species. The highest average seedling height, stem girth, number of leaves, leaf length, leaf width, number of roots and root length were not significantly different between *Dasagavya* and *Beejamrita* treated seedlings. However, all these parameters were greater than the control. The highest seed vigor index was recorded in *Dasagavya* treated seedlings of all vegetable species while the lowest was in the control treatment. However, the Seed Vigor Index (SVI) of *C. melo, L. acutangula*, *B. oleracea* and *S. lycopersicum* were not significantly different among *Dasagavya* and *Beejamrita* treated seeds. Based on the results, it can be suggested that the *Dasagavya* and *Beejamrita* could be effectively used as organic seed primers and liquid fertilizers for seedlings of eight vegetable species evaluated in the present study. Furthermore, *Dasagavya* and *Beejamrita* have exhibited the potential of escalating germination and early seedling growth through hydro priming.

Keywords: *Beejamrita*; *Dasagavya;* Organic liquid preparation; Seeds; Seedlings

Introduction

Seedling growth is modulated by cell division, expansion and differentiation through cell biochemistry and physiology (Kalve *et al.*, 2014). The secondary cells begin the vacuolation and enlarge with the growth of the meristem (Cosgrove, 2000). Seed germination is an intricate procedure entailed with different metabolic activities that resulted in radical and plumule emergence. Stored food reserves in the seeds are used for this purpose (Pritchard *et al.*, 2002). Unfavorable environmental conditions and abiotic stresses are the major issues affecting seed germination, seedling vigor and ultimately on crop yield (Khalid *et al.*, 2019). These factors can reduce plant growth rate, delay seeds germination and impact on soil biota, resulting in massive economic loss. Agricultural countries like Sri Lanka impulse the need for simple and cost effective technologies to enhance the seedling establishment under different environmental conditions (Pawar and Laware, 2018).

Seed treatments reduce the excessive use of fertilizers and soil amendments while improving crop yield by synchronizing seed germination and seedling growth (Mavi *et al.*, 2006). Seed treatments are done before sowing involve: hydration of seeds to enable metabolic activities prior to the germination (Lutts *et al.*, 2016). Seed treatment is an approach that involves nursing seeds with different organic or inorganic compounds for a specified time period under controlled conditions (Chormule *et al.*, 2018). This stimulates various metabolic processes in seeds that improve germination rate in several seed species, including vegetables, grasses and ornamental species while reducing the detrimental outcomes of seed deterioration (Afzal *et al.*, 2016). Application of *Dasagavya* and *Beejamrita* as a seed treatment consider as a cheap, easy, effective and low-risk technique.

Treated seeds have enormous benefits including uniformity in growth, early and faster germination with optimum vigor (Abinaya *et al.*, 2020). Seed treatments further enhance the water use efficiency, roots growth and germination of dormant seeds by increasing metabolic processes, initiate the growth of reproductive organs and early flowering (Farooq *et al.*, 2019). Mainly, treated seeds compete with weeds and combat against the biotic and abiotic stresses (Harris*et al.*, 2002). Generally, the seeds undergo three phases in germination (Bradford, 2002) and during seed priming, the first and second stages undergo with several metabolic and translation processes but not the third stage of germination (Hussain *et al.*, 2016). With this sequence, many benefits are levied to seeds for increasing the growth and germination rate. Hence it reduces the time needed for cellular activities and Deoxyribonucleic acid synthesis in seeds (Yu *et al.*, 2015). This leads to have many biochemical changes in seeds including starch metabolism (Waqas *et al.*, 2019).

Improper use of synthetic agro-chemicals resulted in manifesting adverse effects on the environment, soil and groundwater. Reduction of soil quality is becoming ubiquitous and adversely impacts sustainability, quality of food and nutritional security (Vasu *et al.*, 2020). Insatiable use of agricultural inputs has led to developing resistance to pests and diseases while destroying beneficial ecosystem dynamics. Diminishing favorable densities of pollinators, parasitoids and predators due to the use of inappropriate quantities of agrochemicals were affected on food productivity and food safety (Losey *et al.*, 2006). In organic farming, there is a lack of quality input to be used in seeds and nursery management (Dedefo *et al.,* 2017). *Dasagavya* and *Beejamrita* are two key components of organic farming and emerged as cost effective biological enhancers (Ram and Pathak, 2019). Drawing farmer's attention to organic farming was exorbitant as it is a sustainable farming system with zero chemicals and can regain ecosystem prosperity (Naikwade *et al*., 2012).

Cow dung and cow urine are well known as pools of nutrients while *Dasagavya* and *Beejamrita* are derivatives of cow excreta. *Dasagavya* and *Beejamrita* are techniques of seed treatments designed from locally available raw materials and the use of these organic treatments is well recommended for seeds than synthetic treatments (Ram, 2019). Seeds treated with poisonous fungicide can destroy useful and effective microbes living in the rhizosphere (Shahid *et al.*, 2019). Furthermore, poisonous chemicals and synthetic compounds are absorbed by the roots and deposited in various tissues of the plant leading many implications (Shahid *et al.*, 2017). *Dasagavya* was composed of several medicinal plant extracts together with *Panchagavya*. Due to the combination effects of medicinal plants extracts, *Dasagavya* exhibited a relatively high level of insecticidal properties. There is 5% mortality at 10% concentration of *Dasagavya* in diamondback moth and aphids (Chandrashekharaiah *et al.*, 2013).

Artemisia nilagirica, Leucas aspera, *Lantana camera*, *Datura metal* and *Phytolacca dulcamera* plant species are recommended for temperate countries as these species are abundant in the regions. However, Neem (*Azadirachta indica*), *Calotrophis gigantia*, *Pongamia pinnata, Tephrosia purpurea*, *Jatropha curcas*, *D. metel*, *Adathoda vasica* and *Vitex negundo* plant species are recommended in tropical areas (Selvaraj, 2006*).* The *r*egular use of *Dasagavya* preparation effectively controls a wide range of pests and diseases including: leaf spot, powdery mildew, blight and rust (Kumaranag *et al.*, 2013). Treated plants with 3% concentration of *Dasagavya* manifested inhibitory effects against aphids, thrips, whiteflies, mites and caterpillar (Selvaraj, 2006). *Beejamrita* is a conventional biofertilizer that used to treat seeds which enhance seed germination, avoid phytopathogenic infections and improve seedling vigor (Palekar, 2006). It has the capability to protect the crop from detrimental soil and seed-borne diseases during the early seedling stage. *Beejamrita* showed anti-bacterial, anti-fungal and anti-viral properties against crop diseases (Vyankatrao, 2019). Preparations of these bio-enhancers are very cheap and easy for small-scale and resource poor marginal farmers. Hence, the present study was deliberated to compare the impact of *Dasagavya* and *Beejamrita* as organic seed treatment and growth enhancer at early seedling stage of different vegetable varieties commonly grown in Sri Lanka.

Materials and Methods

Two organic seed primers, *Dasagavya* and *Beejamrita*, were used as the treatments along with the control treatment of water. The present experiment was conducted in sand trays and black color pots from July to October 2021. The average daily temperature, relative humidity and mean rainfall of the area were 32 °C, 73% and 1165 mm, respectively. This experiment consisted of eight sub-experiments, each for a vegetable species, which was carried out with four replicates and setup as a completely randomized design. Eight vegetable species such as: okra (*A. esculentus*) variety (var) MI5, winged bean (*P. tetragonolobus)* var. Green, Chinese spinach (*A.* spp) var. Red, cooking melon (*C. melo)* var. Mal, ridge gourd (*L. acutangula*) var. Local, cabbage (*B. oleracea*) var. *Jambo*, brinjal (*S. melongena*) var. *Lenairi,* tomato (*S. lycopersicum*) var. Local were used as test crops and 120 seeds from each species were used.

The *Dasagavya* was prepared by using healthy leaves of plant species of *A. indica (Kohomba), C. gigantia* (wara), *V. negundo (nika), T. purpurea (pila), J. curcas (edaru), D. metel (aththana)*, *P. pinnata (magul karada)* and *A. vasica (adathoda).* The collected leaves were cleaned properly before obtaining the leaf extract. 1 kg of matured leaf samples from each species was separately soaked in 1 L of cow urine for ten days. After that, 1 L of each filtered extract was added to 5 L of the *Panchagavya* preparation. The mixture was again kept for another 25 days for fermentation. During that period the mixture was stirred well to ensure thorough mixing of *Panchagavya* and the eight plants extracts (Chandrashekharaiah *et al.*, 2013). *Panchagavya* was prepared using 500 g of fresh cow dung, 300 mL of cow urine, 200 mL of curd, 200 mL of cow milk and 100 mL of ghee (Ali *et al.*, 2011; Athavale *et al.*, 2021). *Dasagavya* should filter well before applying to plants and 3% concentration was recommended as foliar sprays and seeds treatments. All seed samples were soaked in *Dasagavya* for 20 minutes before sowing.

Beejamrita was composed of farmyard waste materials: cow dung, cow urine, topsoil and lime powder (Shyamsunder, 2021). First, 2.5 kg of fresh cow dung collected from a native cow was wrapped in a cotton cloth and hang in a 10 L water bucket for 12 hours. Then 2.5 L of cow urine collected from the native cattle, a handful of topsoil and 25 g of lime powder were added to 10 L of water and stirred well. Seed lots were wrapped in a cotton cloth prior to soak in *Beejamrita* solution at the 7th day of the preparation. Generally, newly prepared fresh *Beejamrita* was used for seed treatment but, microbial analysis of *Beejamrita* showed that, it is the best to be used on the 7th day of preparation (Ram and Pathak, 2019). The solution was stirred in a clockwise direction before using for seed treatments. Wrapped seed lots were dipped in *Beejamrita* solution and placed there for 20 minutes until whole seed lots get completely soaked.

Finally, soaked seeds were air-dried in a shade place without exposing to the direct sunlight and rain. Air drying brings back them to their original moisture content, hence radicles were not appearing before sowing. Similarly, coating and combining or mixing *Beejamrita* can be practiced by hands during seed treatments. A similar procedure was adhered by Shyamsunder (2021) with the use of 20 L of water, 5 L of cow urine, 5 kg of cow dung, 50 g of lime powder and a handful soil for the preparation of *Beejamrita*. Under control treatment, all seed samples were soaked in clean running water for 12 hours while wrapping in a cotton clothes.

Treated seeds were sown in washed-fine sand trays with optimum moisture. Randomly selected ten seeds were sown in each sand tray after been treated with *Dasagavya, Beejamrita* and water separately. Daily germination was noted for 10 days and seeds having 2 mm radical emergence was considered as germinated seeds. The Final Germination Percentage of seeds (FGP; number of germinated seeds per total number of seeds sown and present as %), Mean Germination Time (MGT; equation 1), GI (equation 2), and Mean Daily Germination (MDG; ratio between final germination percentage and total number of days) were calculated (Ekanayake and Fernando, 2021). Similarly, a standard equation for SVI was used to find the vigor of eight vegetable species (Williamson and Richardson, 1988) (equation 3).

$$MGT = \frac{\sum_{i=1}^{k} NiTi}{\sum_{i=1}^{k} Ni} \qquad \text{Equation 1}$$

Ni = number of germinated seeds at the i^{th} time (not the cumulative number, Ti = time taken from the beginning to the i^{th} observation, only take the corresponding number relevant to the i^{th} observation), k = last time of seeds germination

$$GI = N1/T1+N2/T2+N3/T3+....+Nn/Tn \qquad \text{Equation 2}$$

N1, N2, N3,……, Nn = number of germinated seeds at a time (days)
T1, T2, T3, …..,Tn = number of germinated seeds at a specific time (not the cumulative number)

$$\text{Seed Vigor Index (SVI)} = \frac{Germination\ percentage \times Seedling\ length\ (Root+Shoot)}{100} \qquad \text{Equation 3}$$

All germinated seeds were transferred to black color pots with 10 cm of diameter filled with the potting mixture prepared by mixing sand: topsoil: coir dust: compost in 1:1:1:1/4 ratio (v/v). Early seedling growth was examined after 15 days of germination. 50 mL of *Dasagavya* and *Beejamrita* were applied at 3% concentration once per two days. Average seedling height, stem girth at 5 cm above the soil level, number of leaves, number of adventurous roots, root length per seedling and leaf length and width were recorded at the end of 15^{th}day. SAS software version 9.1 was used to perform ANOVA for all measured parameters. Means were compared at 5% probability level using Duncan's Multiple Range Test.

Results and Discussion

The FGP of vegetable species, except cabbage, was significantly higher in T1 compared to control treatment (P<0.05; Table 1). The FGP was significantly different among *Dasagavya* and *Beejamrita* treated seeds of okra and winged beans. *Dasagavya* was identified as a biofertilizer and a pesticide rather than a seed primer and, current literature lacks information on *Dasagavya* as a seed treatment. *Panchagavya* was well recommended as a seed treatment to promote seed germination and it was included in *Dasagavya*. Hence, *Dasagavya* also recorded comparatively higher seeds germination percentage. Kumaravelu *et al.* (2009) reported that, the 4% of *Panchagavya* stimulated the germination of green gram while Srimathi *et al*. (2013) revealed that, *Panchagavya* at 2% and 5% with the 8–16 hours soaking gave superior seed germination in biofuel crops like *J. curcas* and *P. pinnata*. *Beejamrita* was considered as an enhancer of plant growth and, plays important role in seed germination and rejuvenation. According to Vyankatrao (2019), 100% *Beejamrita* showed high germination, seedling development and seed vigor index in legume seeds. *Beejamrita* provides excellent strength during the seed germination stage and gives an extraordinary strength for saplings to withstand as individual plants (Smith *et al.*, 2020).

Similarly, the GI was not significantly (p>0.05) different among *Dasagavya* and *Beejamrita* treatments in cooking melon, ridge gourd, cabbage, brinjal and tomatoes. But the GI was significantly different among *Dasagavya* and *Beejamrita* treated seeds of okra, winged beans and Chinese spinach seeds. Moreover, MDG was not significantly different among *Dasagavya* and *Beejamrita* treatments of okra, winged bean, ridge gourd and tomato. The MDG of Chinese spinach, cooking melon, cabbage and brinjal seeds treated with *Dasagavya* and *Beejamrita* were significantly different. In contrast, *Dasagavya* and *Beejamrita* showed the lowest MGT as they showed the fastest germination in okra, winged bean, Chinese spinach, cooking melon, cabbage, brinjal and tomato. Ridge gourd is the only vegetable species which showed significantly different MGT for *Dasagavya* and *Beejamrita* treated seeds.

Table 1: Effect of different seed treatments on FGP, GI, MGT, MDG of vegetable seeds

Vegetable varieties	Parameters	T1	T2	T3	CV%
Okra	FGP	95.0[a]	82.5[b]	70.0[c]	7.8
	GRI	4.35[a]	3.85[b]	3.00[c]	6.4
	MGT	2.13[b]	2.20[ab]	2.45[a]	7.2
	MDG	31.65[a]	27.53[a]	20.40[b]	10.1
Winged bean	FGP	92.5[a]	80.0[b]	67.5[c]	7.8
	GRI	4.33[a]	3.73[b]	2.93[c]	8.6
	MGT	2.18[b]	2.18[b]	2.48[a]	5.9
	MDG	30.83[a]	26.68[a]	19.78[b]	11.8
Chinese spinach	FGP	77.5[a]	65.0[ab]	52.5[b]	13.1
	GRI	3.45[a]	2.75[b]	2.00[c]	10.2
	MGT	2.27[b]	2.50[b]	2.83[a]	6.6
	MDG	25.83[a]	19.15[b]	13.95[b]	17.4
Cooking melon	FGP	90.0[a]	80.0[a]	67.5[b]	9.2
	GRI	2.58[a]	2.30[a]	1.88[b]	8.6
	MGT	3.58[a]	3.53[a]	3.75[a]	3.7
	MDG	22.50[a]	20.00[b]	14.25[c]	8.1

Vegetable varieties	Parameters	T1	T2	T3	CV%
Ridge gourd	FGP	95.0[a]	92.5[ab]	80.0[b]	9.0
	GRI	4.25[a]	3.95[a]	3.18[b]	9.1
	MGT	2.30[c]	2.43[b]	2.65[a]	1.8
	MDG	31.65[a]	30.83[a]	23.13[b]	9.3
Cabbage	FGP	97.5[a]	97.5[a]	90.0[a]	6.6
	GRI	4.83[a]	4.65[a]	3.90[b]	7.3
	MGT	2.03[b]	2.13[b]	2.38[a]	3.8
	MDG	44.58[a]	32.48[b]	30.00[b]	13.7
Brinjal	FGP	100[a]	95.0[a]	82.5[b]	6.9
	GRI	1.83[a]	1.70[a]	1.48[b]	6.8
	MGT	5.48[b]	5.60[ab]	5.70[a]	1.5
	MDG	16.63[a]	14.65[b]	11.80[c]	5.8
Tomato	FGP	97.5[a]	92.5[a]	70.0[b]	9.0
	GRI	2.20[a]	2.05[a]	1.55[b]	9.4
	MGT	4.53[a]	4.58[a]	4.60[a]	2.7
	MDG	19.50[a]	18.50[a]	12.93[b]	11.6

T1 – Dasagavya, T2 – Beejamrita, T3 – control/water, means with similar letters in each row are not significantly different from each other in α=0.05

The highest seedling height, stem girth, leaf length, leaf width, root length and the number of adventurous roots were recorded in *Dasagavya* treatment in cabbage and brinjal seedlings while, the lowest values for above growth parameters were observed in the control treatment (Table 2). The seedling height, stem girth, the number of leaves, leaf width, root length and number of adventurous roots of okra was not significantly different among *Dasagavya* and *Beejamrita* treatments. However, all these parameters were significantly greater than the control treatment. Further, the seedling height, stem girth, number of leaves, leaf width and root length, of winged beans and ridge gourd were not significantly different among *Dasagavya* and *Beejamrita* treatments. In Chinese spinach, the seedling height, stem girth, the number of leaves, leaf length, leaf width and root length were not significantly different in *Beejamrita* and *Dasagavya*. In cooking melon, all the growth parameters were not significantly different in *Dasagavya* and *Beejamrita* treatments. In cabbage, stem girth, the number of leaves, leaf length, leaf width, root length and the number of roots were not significantly different in the first and second treatments.

The number of leaves, root length and number of roots were not significantly different among *Dasagavya* and *Beejamrita* treatments in brinjal. In tomatoes, growth parameters: seedling height, stem girth, number of leaves, leaf length and width were not significantly different in *Dasagavya* and *Beejamrita* treatments. *Dasagavya* consists of miraculous effects on plant growth when all ingredients are suitably mixed in correct proportions. Ram (2019) pointed that the *Dasagavya* enriched with fermentative bacteria, *Lactobacillus* and derive various beneficial metabolites including organic acids, hydrogen peroxide and antibiotics, effectively fight against pathogenic organisms. Selvaraj *et al*. (2007) found that *Dasagavya* owned the potential to increase the shelf life, quality, quantity and taste of many fruits and vegetable varieties with high biological efficiency in crops. *Beejamrita* is a rich source of macro and micronutrients and provides protection to the crops from various seed borne diseases while ensures better plant establishment with Total Nitrogen (770 ppm), Phosphorus (166 ppm), Potassium (126 ppm), Zinc (4.29 ppm), Copper (1.58 ppm) and Ferrous (282 ppm) as the main components included in the *Beejamrita* solution (Shyamsunder, 2021)

Table 2: Average seedling height, stem girth, number of leaves, leaf length, leaf width and the number of roots under different seed treatments

Vegetable varieties	Parameters	T1	T2	T2	CV%
Okra	Seedling height (cm)	30.0^{a}	29.35^{a}	28.22^{b}	2.1
	Stem girth (mm)	3.85^{a}	3.68^{a}	3.38^{b}	3.5
	Number of leaves	4.0^{a}	4.0^{a}	3.5^{a}	8.7
	Leaf length (cm)	4.60^{a}	4.33^{b}	3.93^{b}	3.3
	Leaf width (cm)	4.08^{a}	3.95^{a}	3.70^{b}	2.7
	Root length (cm)	9.6^{a}	9.4^{a}	9.08^{b}	1.5
	Number of roots	22.75^{a}	20.25^{ab}	18.0^{b}	9.2
Winged bean	Seedling height (cm)	40.88^{a}	39.98^{a}	38.40^{b}	2.3
	Stem girth (mm)	5.20^{a}	5.03^{ab}	4.63^{b}	5.1
	Number of leaves	6.0^{a}	6.0^{a}	5.5^{a}	5.7
	Leaf length (cm)	5.3^{a}	5.1^{b}	4.83^{c}	1.7
	Leaf width (cm)	4.15^{a}	4.0^{ab}	3.88^{b}	3.5
	Root length (cm)	18.70^{a}	18.40^{a}	17.95^{b}	1.4
	Number of roots	18.5^{a}	16.75^{b}	15.5^{c}	3.3
Chinese spinach	Seedling height (cm)	11.35^{a}	11.13^{a}	10.70^{a}	3.6
	Stem girth (mm)	1.00^{a}	0.93^{a}	0.78^{b}	10.1
	Number of leaves	4.0^{a}	4.0^{a}	3.5^{a}	8.8
	Leaf length (cm)	2.13^{a}	2.05^{a}	1.80^{b}	4.0
	Leaf width (cm)	1.50^{a}	1.38^{a}	1.15^{b}	7.8
	Root length (cm)	6.13^{a}	5.95^{ab}	5.75^{b}	2.9
	Number of roots	4.5^{a}	3.5^{b}	3.0^{b}	12.9
Cooking melon	Seedling height (cm)	26.20^{a}	26.03^{ab}	25.80^{b}	0.7
	Stem girth (mm)	3.60^{a}	3.50^{a}	3.20^{b}	2.4
	Number of leaves	4.0^{a}	4.8	3.5^{a}	8.7
	Leaf length (cm)	4.55^{a}	4.45^{a}	4.28^{b}	2.5
	Leaf width (cm)	5.08^{a}	4.95^{a}	4.73^{b}	1.7
	Root length (cm)	5.08^{a}	4.95^{a}	4.73^{b}	1.7
	Number of roots	29.0^{a}	27.25^{ab}	25.75^{b}	4.8
Ridge gourd	Seedling height (cm)	25.4a	24.88^{ab}	24.30^{b}	2.3
	Stem girth (mm)	5.03^{a}	4.83^{a}	4.53^{a}	6.5

	Parameters	T1	T2	T2	CV%
	Number of leaves	7.0^a	7.0^a	6.5^a	4.9
	Leaf width (cm)	7.00^a	6.75^{ab}	6.48^b	2.7
	Root length (cm)	7.45^a	7.23^a	6.95^b	2.3
	Number of roots	36.0^a	32.5^b	28.25^c	5.0
Cabbage	Seedling height (cm)	9.35^a	9.13^b	8.78^c	1.5
	Stem girth (mm)	1.05^a	1.05^a	0.90^b	8.16
	Number of leaves	3.0^a	3.0^a	2.5^a	11.8
	Leaf length (cm)	1.08^a	0.98^a	0.85^a	14.0
	Leaf width (cm)	1.05^a	0.98^{ab}	0.80^b	14.1
	Root length (cm)	4.28^a	4.13^{ab}	3.98^b	3.6
	Number of roots	12.75^a	12.75^a	10.5^b	10.0
Brinjal	Seedling height (cm)	7.18^a	6.48^b	6.08^c	3.2
	Stem girth (mm)	1.20^a	1.00^b	0.75^c	12.2
	Number of leaves	3.5^a	3.5^a	3.0^a	14.1
	Leaf length (cm)	2.58^a	2.20^b	1.90^c	3.9
	Leaf width (cm)	1.53^a	1.35^b	1.08^c	8.2
	Root length (cm)	4.15^a	3.95^a	3.58^b	3.7
	Number of roots	13.75^a	12.25^a	10.5^b	7.9
Tomato	Seedling height (cm)	11.90^a	11.63^a	9.85^b	6.0
	Stem girth (mm)	1.08^a	1.0^{ab}	0.90^b	8.7
	Number of leaves	4.0^a	3.75^a	3.5^a	11.8
	Leaf length (cm)	2.00^a	1.88^a	1.58^b	7.2
	Leaf width (cm)	1.08^a	0.98^a	0.70^b	8.5
	Root length (cm)	4.68^a	4.38^b	4.10^c	3.5
	Number of roots	12.75^a	10.25^b	8.25^b	14.4

T1- Dasagavya, T2- Beejamrita, T3- control/water, means with similar letters in the same row are not significantly different from each other in α=0.05

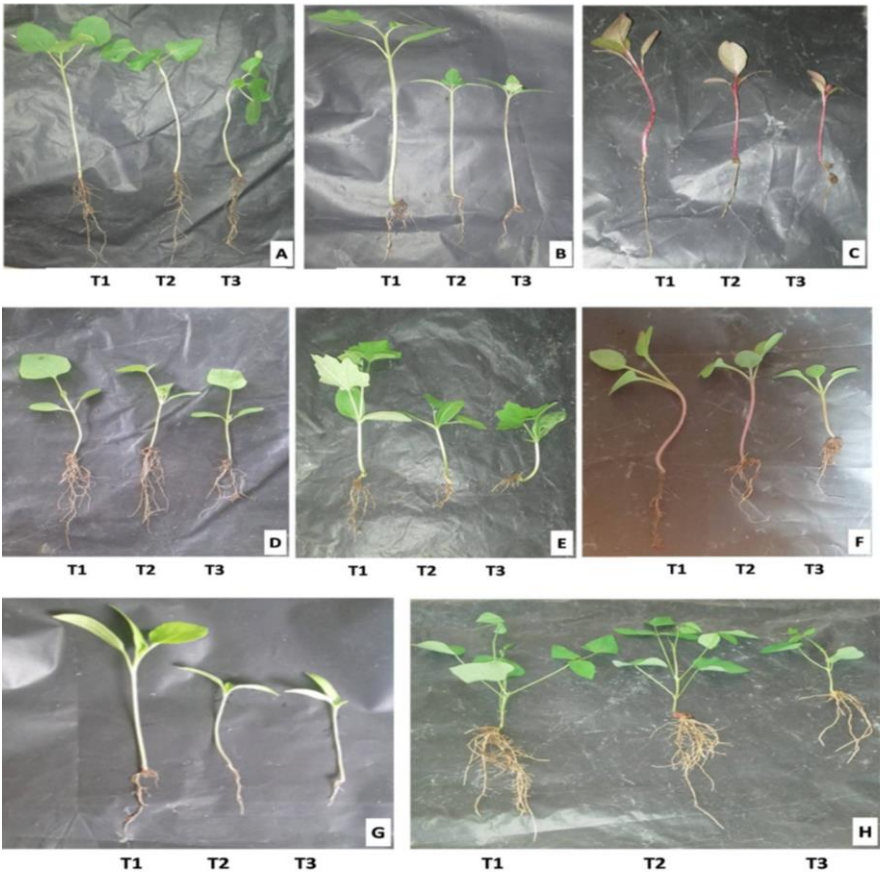

Plate 1: Effect of different seed treatments on growth of seedlings (A) okra; (B) tomato; (C) Chinese spinach; (D) Cooking melon (E); ridge gourd (F); cabbage (G); brinjal (H) and winged bean (T1- *Dasagavya*, T2- *Beejamrita* and T3- control/water)

Significantly higher ($p<0.05$) seed vigor index was observed in *Dasagavya* treated okra, winged bean, Chinese spinach and brinjal while the lowest seed vigor index was reported in control treatment for all vegetable species (Figure 01). Vyankatrao (2019) found that 100% *Beejamrita* treatment showed the highest percentage of germination, seed vigor index and seedling growth in ground nut, soybean, moth bean and green gram. Further, it has been emphasized that *Beejamrita* was recommended for organic agriculture as its regular use as a seed treatment can alleviate dependence on chemicals and synthetic materials. *Beejamrita* is well known for root protection and strengthening the root system during transplanting (Padmavathy and Poyyamoli, 2011).

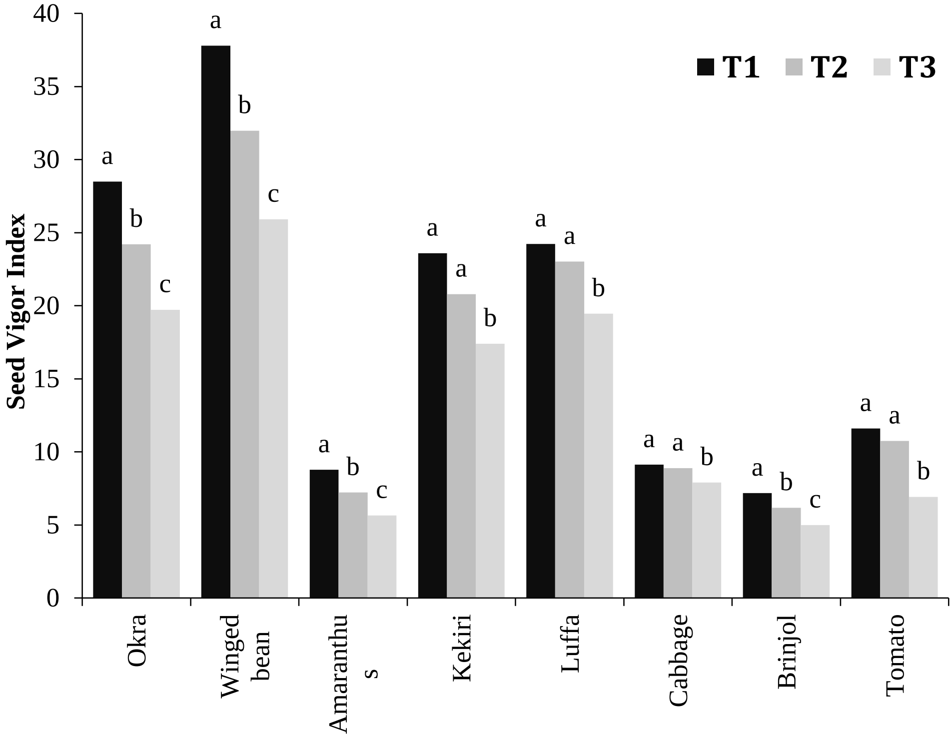

Figure 1: Seed vigor index of eight vegetable species under different seed treatments (T1 - *Dasagavya*; T2 – *Beejamrit;* T3 – control/water), Means with similar letters on the bar are not significantly different from each other in α=0.05 for a given vegetable species

The secondary plant metabolites in *L. camara*, *D. metal*, *Calotrophis* and other herbs highly contributed to the insecticidal properties in *Dasagavya* (Chandrashekharaiah *et al.*, 2015). *Dasagavya* is a growth promoter while it has the potential in boosting immunity against pests and diseases in plants (Pathak and Ram, 2013). Moreover, there is huge number of positive effects of *Dasagavya* on hill crops. Thrips and powdery mildew of rose and gerbera were effectively controlled by applying *Dasagavya* (3%) solution while blister blight disease in tea plantations was controlled by regular application of 3%

Dasagavya at 15 days (Selvaraj, 2006). Moreover, beneficial microbes in *Beejamrita* protect the crop from soil-borne pathogens and avoid seed borne diseases mainly during the seedling stage. It exhibits vast scope of properties: fertilizer, pesticide and manure (Ahilandeswari and Maheswari, 2018).

There were no disadvantages or consequences recorded on seeds, soil and a crop from *Beejamrita* as it is an organic formulation. Supporting our findings, Devakumar *et al*. (2008) and Sreenivas *et al.* (2010) observed that the *Beejamrita* consists of numerous valuable microorganisms including: actinomycetes, phosphorus solubilizers, nitrogen fixers and beneficial fungi that can positively influence on growth and yield of plants. Khan *et al.* (2020) emphasized that, the presence of Abamectin, Bifenthrin, and Difenoconazole commonly used as pesticides and seed treatments are crossing the thresholds in fruit and vegetable samples and, consumers are under possible health risks around the world due to the presence of these synthetics. Biological seed treatment techniques are expected to be growing rapidly in large scale cultivations in the present era due to their environmental friendly nature (Sharma *et al.*, 2015). One of the main limitations of efficient nursery management is the lack of awareness of seed treatments and early seedling among workers. Therefore, it is essential to make extra efforts at farmer level to adapt new organic technologies.

Conclusion

Organic seed treatments and biofertilizers can be effectively utilized to enhance the seed germination and seedling growth at early stages of life of different vegetable species. *Dasagavya* and *Beejamrita* seed treatments were reported to have better seed germination and fast germination compared to the control. *Dasagavya* applied seedlings of okra, winged bean, Chinese spinach and brinjal showed vigorous seeding growth. *Dasagavya* and *Beejamrita* exhibited the potential to be used as seed primers for intensifying germination and seedling growth than water soaking for the vegetable species examined in the present study.

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Evaluation of the Potential of Fungal Acid Proteases of *Aspergillus flavus*, *Aspergillus niger* and *Penicillium* sp. to Produce Shrimp-Waste Protein Hydrolysates with Degraded Antigenic Proteins

M. Gamage[1, 2*], N. Liyanage[3], I. Pathirana[4], S. Ediriweera[5] and L. Prabodha[6]

[1]*Faculty of Graduate Studies, University of Ruhuna, Sri Lanka*
[2]*Diabetes Research Unit, Department of Clinical Medicine, Faculty of Medicine, University of Colombo, Sri Lanka*
[3]*Department of Food Science and Technology, Faculty of Agriculture, University of Ruhuna, Sri Lanka*
[4]*Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka*
[5]*Department of Bio System Technology, Faculty of Science, University of Ruhuna, Sri Lanka*
[6]*Department of Anatomy, Faculty of Medicine, University of Ruhuna, Sri Lanka*

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Abstract

The waste produced by the shrimp industry can be a great source for the recovery of important bioactive compounds with potential applications in food industry. The present study aimed to characterize extracted proteases of *Aspergillus flavus*, *Aspergillus niger* and *Penicillium* sp. to be utilized as potential sources to produce shrimp waste protein hydrolysates with degraded antigenic proteins. The extracellular fungal acid protease enzymes were extracted under solid substrate fermentation and partially characterized the enzyme activities,

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*Corresponding author
Postal address: Faculty of Graduate Studies, University of Ruhuna, Sri Lanka
Email: manojakwije@gmail.com

concerning the reaction temperature and pH of the reaction medium. Then, the capabilities of extracted fungal protease enzymes to reduce the potential allergens of shrimp waste protein hydrolysates were examined by SDS-PAGE. After incubation of all three fungal species at 37 °C and pH 3.0 for 5 days, the highest protease yield, 527.40 μg/mL was achieved from *A. flavus*. The optimum temperature for *A. flavus* for the highest protease activity was 45 °C, while for other two microorganisms the optimum temperature was 50 °C. The optimum pH values for all three proteases were pH 3. According to the SDS-PAGE protein hydrolyzing patterns, the crude acid protease extracted from *A. flavus* was the best protease to degrade the potential antigenic proteins over the other two acid proteases extracted from *A.niger* and *Penicillium* sp., while all three enzymes had shown the potential to reduce the allergenic capacity of shrimp waste proteins through enzymatic hydrolysis.

Keywords: *Aspergillus flavus*; *Aspergillus niger*; Fungal protease; *Penicillium* sp.; Shrimp waste; Solid-state fermentation

Introduction

Proteases account for nearly 60% of the global enzyme industry due to the diverse range of commercial applications in many industries (Rao *et al*. 1998). Fungi species are highly exploited since they produce varied extracellular enzymes and are able to grow on low-cost substrates (Gupta *et al*., 2002). The fungal extracellular protease enzymes are reported to be extracted as acid, alkaline and neutral proteases. The acid proteases are extensively utilized in the food industry (Mukhtar, 2009). Shrimp solid wastes, head, shell and tail portions, have generated many environmental problems. Traditional waste treatment methods are based on chemical techniques which cause environmental pollution, depletion of resources and upsurge the associated costs. Better economic use of shrimp waste would minimize the environmental problems and generate added profits for the industry.

As reported by FAO and WHO, crustaceans are one of eight main allergenic foods and, the crustacean allergic cases have been increased annually (Khanaruksombat *et al*., 2014). The shrimp muscle protein tropomyosin (34-38 kDa), is identified as the most important heat-stable allergen. Antigenic proteins are extensively studied on shrimp muscle though studies on shrimp waste portions were under-discussed. Consequently, it is important to produce shrimp waste with degraded potential antigenic proteins for future applications in the food industry. Therefore, the present study aimed to produce and characterize the fungal proteases extracted from *A. flavus*, *A. niger,* and *Penicillium* sp. as potential sources to produce shrimp waste protein hydrolysates with degraded antigenic proteins.

Materials and Methods

Materials

Three fungal species, *A. flavus*, *A. niger,* and *Penicillium* sp. which were identified under standard protocols during a preliminary study were applied in the present experiments. *Penaeus monodon* including the head, shell and tail were purchased from local market and stored at freezing temperature (-18 °C). The raw white *samba* rice of commercial-grade was purchased from the local market. The standard method described by Lakshman *et al.,* (2010) was followed to produce the fungal protease enzymes by solid-state fermentation. The raw white *samba* rice was used as the solid substrate based on the standard protocol followed by Lakshman *et al.,* (2010) using polished rice for the similar purpose.

Preparation of solid substrate for fungal culture

The solid-state fermentation was conducted to produce enzyme by soaking raw *samba* rice for 18 hours in 500 mL Erlenmeyer flasks. The 30 g substrates were put into the Erlenmeyer flasks, and then, they were plugged with cotton wool. Then they were sterilized at 121 °C for 20 min in an autoclave. The Erlenmeyer flasks were allowed to cool to room temperature.

Solid-state fermentation of fungal culture

The soaked *samba* rice was inoculated with the fungal spore suspensions. Firstly, sterilized 0.005% Dioctyl sodium sulphosuccinate (10 mL) was added to fungal culture. Then the fungal spores were scratched using an inoculating needle. In order to prepare the homogenized spores suspensions, the test tubes were shaken thoroughly. Then 1 mL each from the homogenized spore suspensions of *A. lavus, A. niger* and *Penicillium* sp. were transferred to the pre-sterilized Erlenmeyer flasks which contained soaked *samba* rice. Then they were incubated at the optimum temperature and pH level in the fermentation media for 5 days. The flasks were shaken two times per day and the fermentation was triplicated.

Extraction of fungal acid protease enzymes

On the fifth day of incubation, the fermented rice was mixed with citrate/phosphate buffer (pH 3) in the ratio of 30 mL of buffer with 10 g of rice, and the resultant mixture was kept at 4 °C while shaking for 1 hour. Then it was filtered using a Whatman filter paper No. 44, and the filtrate was centrifuged at 3000 rpm for 15 minutes at 4 °C. The crude enzyme was collected from the supernatant.

Optimization of fermentation temperature and pH

The protease enzyme production conditions were optimized by incubating the fermentation flasks at different temperatures (20, 28, 37, 45, 50 and 60 °C) at neutral pH for 5 days. Once the optimum temperature was found at neutral pH, the optimum incubation pH was studied at different levels (2.0, 2.5, 3.0, 4.5 and 5.0) for 5 days of incubation. The used buffer systems included, Citrate-sodium phosphate buffer of pH 5.0-7.0, lactate buffer of pH 3.0-3.5 and Sodium acetate buffer of pH 2.0-2.5.

Enzyme activity of extracted proteases

The universal protease activity assay was conducted as explained by Lakshman *et al*., (2010) using casein as the substrate. Firstly 2% casein solution (0.25 mL) was added to vials, and then, they were allowed to equilibrate in a water bath at 50 °C for about 10 minutes. After that, 0.025 mL volume of isolated crude enzyme was added to two of the test sample vials, but not the blank.

After 10 minutes of incubation at 50 °C, the reaction was terminated by adding 0.4 $molL^{-1}$ trichloroacetic acids (0.5 mL). The supernatant was collected by centrifugation (at 3000 rpm for 5 minutes) of the terminated reaction mixture. The colorimetric method was used to determine the liberated amount of tyrosine by adding 5 × *Folin-Ciocalteu's* reagent (0.2 mL) and 0.5 mol L^{-1} Na_2CO_3 (1 mL) to the supernatant (0.2 mL). A spectrophotometer (UV mini 1240, Japan) was used to measure the optical density at 660 nm. The tyrosine standard curve was developed by conducting a tyrosine assay and then, it was used to calculate the amount of released tyrosine. One unit of the acid protease activity was defined as the amount of enzyme that yielded 1 μg of tyrosine per minute at the temperature and pH of the reaction mixture. The protease activity assay was done in duplicates, and the mean values were calculated.

Partial characterization of protease enzyme activity

The optimum temperature for the highest enzyme activity was studied at different temperatures (20, 28, 37, 45, 50 and 60 °C) at neutral pH. The optimal pH for the maximum protease activity was evaluated at a series of pH levels (2.0, 2.5, 3.0, 5.0 and 7.0) at their optimum temperatures.

Production of shrimp waste protein hydrolysates with protease treatment

Shrimp muscle and waste protein hydrolysates were separately prepared by following the methods explained by Limam *et al.*, (2008) with slight modification. Shrimps were washed thoroughly under running water and Shrimp muscle and waste portions (head and shell) were separately minced using a blender, and 100 g of each sample was taken for each hydrolysis. Then, they were mixed with distilled water at the 1:1 (w/v) proportion, and a glass rod was used to shake and homogenize the solution. The incubation period was extended for 1 hour and during this period, it was occasionally stirred. The reaction mixture was kept at 95 °C for 5 minutes to inactivate the endogenous hydrolyzing enzyme. Shrimp proteins were hydrolyzed at the ideal temperature and adjusted the pH of the respective enzymes. The reaction mixture was heated at 90 °C for 5 minutes to terminate the proteolysis. Then, they were kept under the ambient temperature and filtered using a muslin cloth. Then, it was centrifuged at 3000 rpm and 4 °C for 15 minutes and the supernatant was collected. For the explained experiment, 0.5 mL of shrimp protein samples were treated with 0.025 mL of extracted crude enzymes of three fungal species.

SDS PAGE for shrimp waste protein hydrolysate

The standard protocol explained by He, (2011) for SDS-PAGE was conducted to observe the separation of protein bands based on their molecular weights in shrimp waste protein hydrolysate. The polyacrylamide of separation gel (12%) and polyacrylamide of stacking gel (4%) were arranged by the following standard procedures. The protein band patterns of shrimp muscle and waste samples prior to and after treatment were observed through the SDS PAGE analysis. Shrimp protein samples were mixed with Laemmli sample buffer (ratio 2:1) and then, the sample was heated in a water bath (95 °C for 5 minutes). Then, the samples were cooled to ambient temperature. Then the mixtures were centrifuged (3000 rpm and 5 minutes) to settle any precipitated particles prior to being loaded into the wells. Then, 20 µL of each protein mixture was loaded onto wells and the gel was run at 300 V (2 h) until reaches the separating gel bottom. The Coomassie brilliant blue R-250 was used to stain the gels for the detection of the separated protein bands. Finally, the gel was de-stained overnight till the protein bands were visible as dark blue bands against a transparent background. The standard protein marker ranged from 14.4 to 97.4 kDa was used in SDS PAGE as a reference to estimate the molecular weight of separated protein bands.

Results and Discussion

Protease yield

Under solid – state fermentation of all three fungal species, the highest protease yield, 527.40 µg/mL was achieved from *A. flavus* over *A. niger* (234.52 µg/mL) and *Penicillium* sp. (147.60 µg/mL) after 5 days of incubation. However, the present results are not following the findings of Anand. K, (2016) who stated that *A. niger* grown at 37 °C showed the maximum enzyme production over *A. flavus* under submerged fermentation of rice bran. In contrast to the present results, Sethi and Gupta, (2015) had gained a higher alkaline protease activity by fermentation of *Penicillium chrysogenum* than that of *A. niger*.

Effect of temperature and pH on protease production

As presented in the Figure. 1, 37 °C was the optimum temperature since protease production was gradually increased over the elevated incubation temperatures 20, 28, and 37 °C, and thereafter, the enzyme yield of all three fungal species were started to decline with further increase of incubation temperature for all studied fungal species. The higher temperature might reduce the fungal growth, and thus, reduce the enzyme yield as enzyme is a secondary metabolite produced through the exponential growth phase of microorganisms. Although the rates of physiological processes are increased by rising the temperature up to a certain extent, and it starts to diminish the rates due to sensitivity of enzymes at elevated temperatures (Mukhtar, 2009). The present findings are following the results of Anand (2016) who had revealed the highest protease enzyme production at 37 °C under submerged fermentation of rice bran by *A. flavus* and *A. niger*. As evidenced by previous studies, *Aspergillus* species, have a capacity to secrete high levels of enzymes in their growth environment (de Souza 2015).

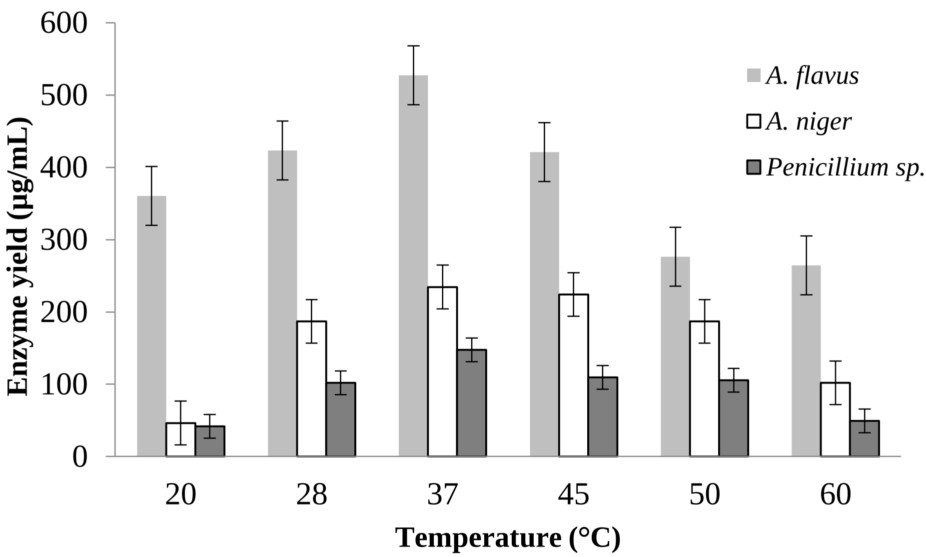

Figure 1: Protease production at different incubation temperatures and neutral pH

As per the basic facts, fungal protease enzyme is a secondary metabolite secreted during their exponential growth phase. The incubation at high temperature could lead to lessen the growth rates, and thus a reduction in enzyme yield could be observed.

Based on the results illustrated in Figure. 2, the highest enzyme yields were observed at pH 3.0 for three fungal species.

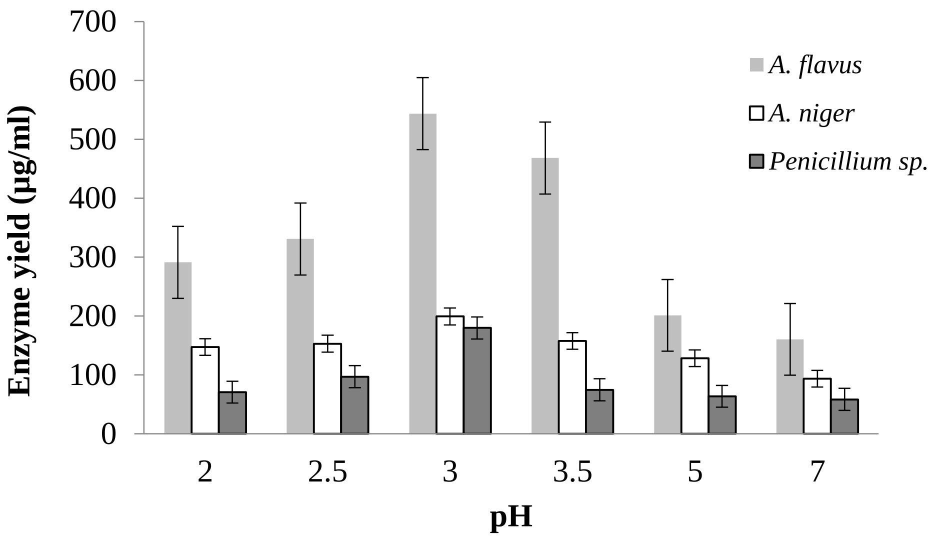

Figure 2: Protease production at different incubation pH levels and optimum temperatures

The pH significantly impacts on the production of enzymes, whereas the variation in pH may cause to denature the enzyme, resulting in loss of catalytic activity. In correspondence with present results, Radha *et al*. (2012) had stated the same figures with *Aspergillus* sp. under the solid – state fermentation of wheat bran. In the controversy with the present findings, Shivakumar (2012) stated that the optimum pH was 5.0 for acid protease production of *A. niger* by wheat bran fermentation, while that was 9.0 in the case of both *P. chrysogenum* and *A. niger* as reported by (Sethi and Gupta, 2015).

Partial characterization of protease enzyme activity

As illustrated in Figure. 3, the optimum temperature for highest protease activity was 45 °C for *A. flavus* enzymes, while that was 50 °C for other two fungal species.

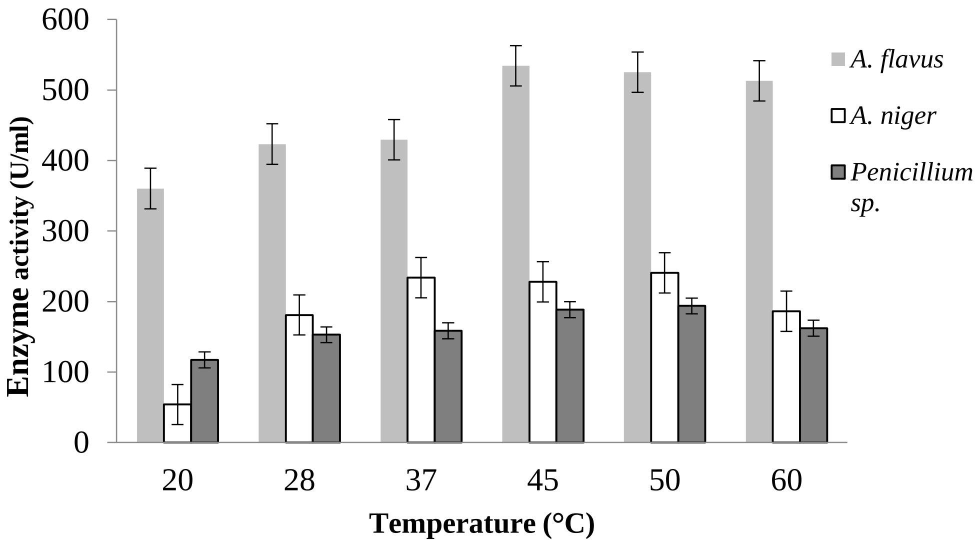

Figure 3: Optimum temperature for the highest enzymatic activity

A recent research article by Anand (2016) has reported that the enzymes produced by submerged fermentation of rice bran by *A. flavus* and *A. niger* were stable at a temperature, ranging from 50 to 60 °C with the gradual increase of enzyme activity with increasing temperature followed by a steep decrease above 60 °C. This result showed that the protease produce by *A. flavus* is a thermo tolerant enzyme which is stable with its structural integrity at above 60 ˚C temperature (Rukmi, 2020). In controversy, Chandrasekaran *et al*. (2015) had stated that, the optimal temperature for protease production by *A. flavus* extracted from paddy soils was 30 °C, while Radha *et al*. (2012) had published that the maximum protease production by *Aspergillus* sp. was observed at temperature 32±2 °C, and at 40 °C. The minimum protease production was noticed. In the argument with the present findings, Shivakumar (2012) has found that the highest protease productivity by *A. niger* was shown at 30 °C. As reported by Sethi and Gupta, (2015), protease activity of *P.chrysogenum* was highest at 35 °C and completely inactivated at 60 °C.

The pH profile of the purified proteases is shown in Figure. 4, and the results revealed that the optimum pH values were similarly aligned at pH 3.0 for all tested proteases.

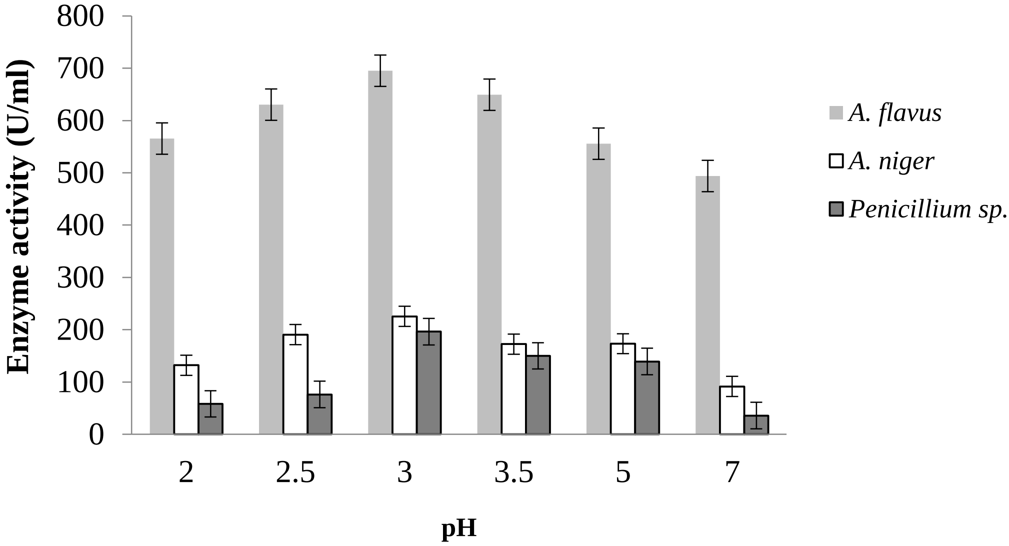

Figure 4: Optimum pH for the highest enzyme activity

These findings are in line with a similar study as mentioned that protease enzymes from *A. niger* were most active at pH 3.0 (Siala *et al*., 2009). However, some controversies were also found in the literature. Tremacoldi *et al*. (2004) indicated that, the optimum pH for *Aspergillus clavatus* protease activity was 2.0, and as reported by Oseni (2011), it was 5.5 for *Penicillium* sp.

SDS PAGE for shrimp proteins

The untreated protein extracts of shrimp muscle, shell and head were profiled by SDS PAGE and the characteristic patterns were obtained for all the extracts of the shrimp samples as illustrated in Figure. 5. There was no observable dissimilarity in patterns of protein bands in between shrimp muscle and shell or head. Four major bands with molecular weights of ~66.2 kDa, ~45 kDa, ~31 kDa and ~21.5 kDa were found in all shrimp protein samples. Literature supports well on the flesh of shrimp, but allergenicity of shrimp shell and head were under-provided.

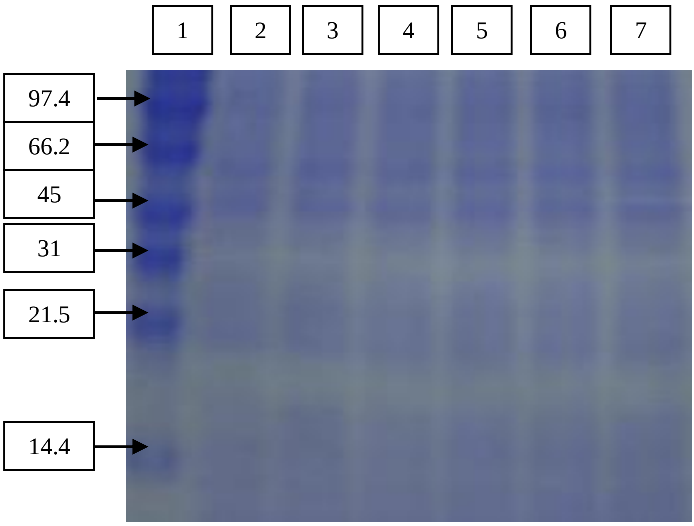

Figure 5: SDS-PAGE for untreated shrimp protein hydrolysate, Lane 1: Protein marker, Lane 2,3: Shrimp muscle, Lane 4,5: Shrimp shell, Lane 6,7: Shrimp head

The major allergens identified from black tiger shrimp were tropomyosin (33kDa), myosin light chain (20 kDa) and argine kinase (40 kDa) (Velickovic and Jankulovic, 2014). All of the samples of shrimp extracts had shown similar bands in the range of 45 – 31 kDa, and the protein bands at ~31 kDa would be the major allergen, tropomyosin ~45 kDa would be the arginine kinase, and ~21.5 kDa supposed to be sarcoplasmic calcium-binding proteins. Several research reviews and articles have mentioned that *tropomyosins* are the cytoskeletal proteins. *Tropomyosins* play a key role in the muscle contraction regulation with other contractile proteins actin and myosin. *Tropomyosins* form head-to-tail polymers along the actin filaments (Stafford *et al.*, 2012). *Tropomyosin* regulates the stability of actin filaments in non-muscle cells (Stafford *et al.*, 2012). These proteins may remain in the derived products from the shrimp waste. Therefore, it is very important to degrade the potential antigenic protein in shrimp waste before further processing.

SDS PAGE for protease treated shrimp waste protein hydrolysate

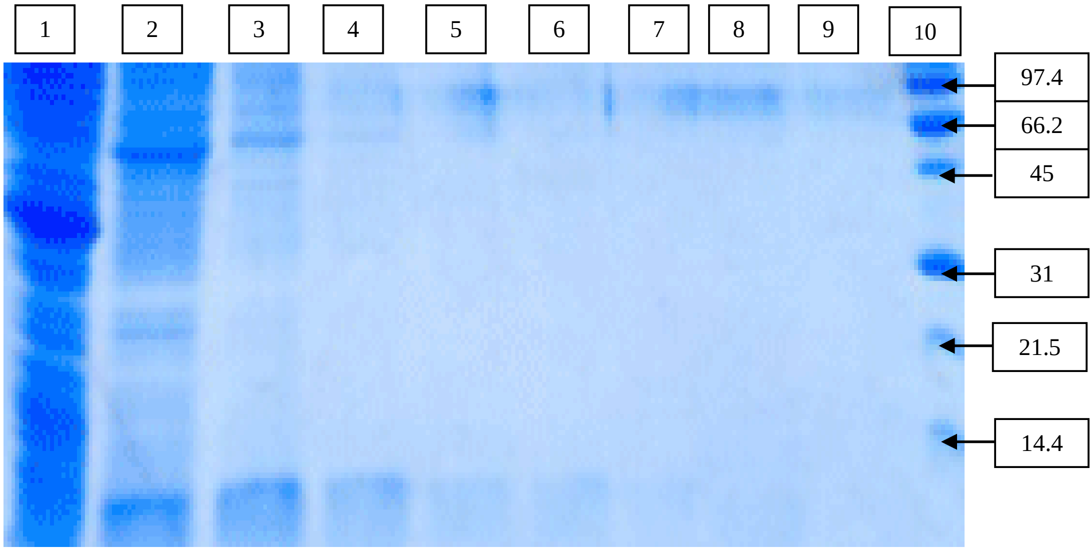

Figure 6: *SDS-PAGE for protease treated shrimp protein hydrolysates* 1-Untreated Shrimp muscle protein extract, 2- Untreated Shrimp waste (head and shell) protein extract, 3-shrimp waste protein hydrolysate treated with mixture of extracted fungal enzymes 4-Shrimp waste protein hydrolysate treated with enzyme extracted by *Aspergillus niger*, 5-Shrimp waste protein hydrolysate treated with enzyme extracted by *Penicillium* sp., 6-Shrimp waste protein hydrolysate treated with enzyme extracted by *Aspergillus flavus,* 7-Shrimp waste protein hydrolysate treated with enzyme extracted by *Aspergillus niger*, 8-Shrimp waste protein hydrolysate treated with enzyme extracted by *Penicillium* sp., 9-Shrimp waste protein hydrolysate treated with enzyme extracted by *Aspergillus flavus,* 10- Protein Marker

As per Figure. 6, the shrimp waste samples treated with three fungal proteases had a great reduction in protein bands compared to the untreated sample proving that the extracted crude acid protease enzymes affected on hydrolyzing the shrimp proteins. According to Figure. 6, lanes 3, 4, 5, 7, and 8 have much darker bands at the bottom (<14.4 kDa) than in lanes 6 and 9, indicating that there is a much greater concentration of smaller peptides because hydrolysis of shrimp waste protein is a process to degrade the long protein chain in shrimp head and shell into small peptides and amino acids. This would suggest that crude acid protease extracted from *A. flavus* does a great degree of hydrolyzing protein than the other two enzymes.

The further increase of enzyme concentrations of none of the enzymes is further effective on hydrolyzing potential antigenic proteins. The literature survey had found many reports with evidence, when the concentration of enzyme added reaches a certain point, the increase of soluble protein in hydrolysate would not increase significantly or even does not increase at all. Similar findings were investigated by Benito *et al*. (2002) who has found that protease extracted from *P. chrysogenum* Pg 222 was effective against the myofibrillar proteins myosin, actin and tropomyosin. Another study Paul *et al*. (2015) has stated that, shell of the black tiger shrimp was a good protein source, while a different recent study has invented that crude alkaline proteases from *Bacillus invictae* could be utilized to degrade the shrimp shell proteins (Hammami *et al*.. 2017). The same kind of investigation has been found after treatment with pepsin (Mejrhit *et al*., 2017). The experiments of Shimakura *et al*. (2005) and Liu *et al*. (2011) have also found the effectiveness of protease to degrade *tropomyosin.*

Conclusion

A. flavus, *A. niger* and *Penicillium* sp. are effective protease producers under solid-state fermentation of white *samba* rice. Their extracellular crude acid proteases have higher proteolytic activity against potential antigenic proteins in shrimp waste. The crude acid protease extracted by *A. flavus* is found to be the best to degrade potential antigenic proteins of shrimp waste over the other two acid proteases extracted by *A. niger* and *Penicillium* sp. Those fungal acid proteases were found to be potential sources to produce shrimp – waste protein hydrolysates with degraded antigenic proteins.

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Impact of Visual and Verbal Elements of Eco-Friendly Packaging on Consumer Buying Behavior

H.M.G.M Bandara, M.G.T Lakmali * and G.C Samaraweera

Department of Agricultural Economics, Faculty of Agriculture, University of Ruhuna, Sri Lanka

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Abstract

Designing an eco-friendly offering for the market needs a thorough understanding of the nature of eco-friendly consumers. However, organizations are merely concerned about the impact of eco-friendly packaging on purchasing behavior of the consumer without considering the main elements demanded by consumers in detail. Hence, organizations spend many resources on eco-friendly packaging without emphasizing the effect of packaging elements on consumer buying decisions that ultimately yield low profits with foreseeable environmental threats. The absence of concrete results published in this regard, especially concerning the Sri Lankan context accelerates this negative impact. Therefore, the present research examines the effect of visual and verbal elements of eco-friendly packaging on consumer buying behavior with special reference to dairy products while considering visual and verbal as the main packaging elements and dairy products as the main product category.

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*Corresponding author:
Postal address: Department of Agricultural Economics, Faculty of Agriculture, University of Ruhuna, Sri Lanka
Email: thushlakmali95@gmail.com

Data were collected by distributing a pre-tested structured questionnaire among the consumers (n = 104) shopping at Cargills Food city outlet, Galgamuwa. Wilcoxon Signed-Rank test results revealed that, visual elements: shape ($p = 0.001$; $t = 5.029$), material ($p = 0.001$; $t = 7.412$), size ($p = 0.001$; $t = 5.029$) and graphics on the package ($p = 0.064$; $t = -1.849$) showed a significant impact on buying behavior while, color ($p = 0.706$; $t = -0.377$) proved insignificant. Moreover, verbal elements including product ($p = 0.000$; $t = 7.771$)) and nutritional ($p = 0.001$; $t = 7.454$) facts showed a highly significant impact on purchasing dairy products with eco-friendly packaging. The Chi-square test revealed a highly significant relationship between purchasing environmentally-friendly packages with educational level ($p = 0.015$; $x^2 = 12.339$) and a marginally significant relationship with the occupation of consumers ($p = 0.064$; $x^2 = 8.900$). Findings of this research direct marketers, and product designers, in particular, to manufacture packages incorporating consumer-preferred visual and verbal elements of packaging to attract consumers towards eco-friendly packed dairy products in particular.

Keywords: Consumer buying behavior; Eco-friendly packaging; Elements of packaging

Introduction

Eco-friendly packaging is the process of creating and designing containers for physical commodities, using materials and procedures which are having the least impact on the environment by utilizing a minimum number of resources and energy. It is a green concept that emerged not more than a decade ago, where environmental sustainability is mainly focused in a world in which the environmental deterioration continues terribly at present. The growing population accelerates this detrimental impact. Discarded packaging material is a very apparent source of trash, causing a serious challenge in waste management (Zhong *et al*., 2020). Polyethylene is the most widely utilized petroleum-based polymer in manufacturing packages (Emadian *et al*., 2017; Singh *et al*., 2017). However, it is incredibly difficult for these types of polymers to be biodegraded after disposal. Therefore, the accumulation of those on land or coast creates varying levels of contamination.

Further, the packaging considers the major accumulated leakage. At present, the volume of plastic waste is ultimately responsible for discarding more than 150 million tons into the ocean (Conservancy, 2015). Unlike some other types of waste, plastic does not decompose (Conlon, 2020). Currently, environmental conservation has become one of the critical issues that can grab the attention of the world population (Esmaeilpour and Rajabi, 2016). For these reasons, sorting the balance between the safety of the product being supplied to the market and the usage of packing material may cause the conservation of natural resource base and minimize environmental deterioration while, enhancing the overall eco-system efficiency (Tiekstra *et al*., 2021). Therefore, past researchers have stressed the need of searching for biodegradable alternatives which are mainly recyclable to lessen the environmental deterioration mainly caused by the disposal of plastic packing materials (Wang and Wang, 2021).

With the emergence of eco-friendly theories and concepts everywhere in the world, companies are focusing more on satisfying consumer needs and want with a minimal impact on both present and future interests of the society. An interesting fact is that, modern consumer is more informative and well aware of their buying behaviors compared to earlier consumers, where modern consumers are very much concerned about the impacts of their purchasing decisions on society (Shabbir *et al*., 2020). Marketers have to switch their business operations targeting these eco-friendly consumer groups. Since marketing strategies should tally with the needs and wants of responsible consumers (Schiffman *et al*., 2015), the consideration of the elements demanded by consumers for eco-friendly packaging is vital to consider. Traditional business operations will not earn them more profits as the consumers are demanding environmental friendly products and services at present (Moravcikova *et al*., 2017). Therefore, organizations need to switch their old conventional business operations to target the emerging needs and wants of the modern responsible consumers.

With the emergence of responsible consumerism concepts across the globe, consumers have adjusted their needs and wants considering the impact of their buying behaviors on the environment they reside in. Even though, the consumer's perspective of eco-friendly packaging concerns the environment and the marketer's perspective is concerned with attracting the emerging eco-friendly consumer market segments for higher margins (Nikitaeva, 2012), companies that are successfully targeting eco-friendly touch points and attitudes of modern consumers earn more competitive advantages.

Designing the best eco-friendly package for a physical product is not an easy task. Therefore, it needs to understand consumer preferences, consumer buying behaviors, consumers' psychological background, consumer characteristics and most significantly the elements of packaging. After understanding consumer behavior, packaging elements should be chosen carefully. Selected elements are combined to create the best design for a physical product highly valued by the consumers (Kotler and Armstrong, 2013). However, selecting the correct set of packaging elements for a product needs an examination of an ample amount of consumer research and market research data.

A package is the last and the best chance to influence consumers, choosing the perfect packaging element combination will design the best overall package for a physical product for better results in the market. The environmental friendly nature of packaging is often implied by the visual characteristics including: the size of the package; color; shape; label and graphics on the package (Magnier and Schoormans, 2015; Pancer *et al*., 2017). Both visual elements and verbal elements of packaging are equally important to be considered when designing an environmentally friendly package. Product facts and nutritional facts are the verbal elements of packaging. These elements should be carefully picked when designing the best product package design (Sultan, 2016). It is essential to study how eco-friendly packaging impacts consumer buying behavior and what packaging attributes should provide more attention when designing a feasible eco-friendly package for a physical product to attract and satisfy emerging needs and wants of responsible consumerism.

Designing products with eco-friendly packages, that is having minimal impact on society is challenging. Therefore, this study focuses on exploring the most preferred nature of packaging material by the consumers for eco-friendly packaging. Conducting research on understanding consumer demand and satisfying their preferences using eco-friendly strategies will be of utmost importance in achieving this target. As explained by Chekima *et al*. (2016), demographic characteristics: age; gender; income and educational levels carry positive or negative influences on the promotion of consumer buying behaviors related to the products with eco-friendly packaging (Chekima *et al*., 2016). Therefore, the relationship between the demographic characteristics of the consumers and purchasing products with environmentally friendly packages will be investigated as a means of designing the best packaging for physical products.

Many organizations spend a lot of resources on research for identifying the effect of product packaging on consumer purchasing decisions without considering packing elements in detail. Therefore, that ultimately yield low profits with the environmental threat. Dairy products are considered essential components of the human diet and therefore, they are consumed daily by all age groups thus showing higher buying frequencies. Therefore, the present research primarily focuses to examine the effect of visual and verbal elements of eco-friendly packaging on consumer buying behavior with special reference to dairy products. Furthermore, research publications related to the role of eco-friendly packing on consumer purchasing behaviors are limited. Hence, the present research tries to bridge the knowledge gap existing in the field with special reference to Sri Lankan consumers.

Materials and Methods

Stimulus response model was used as the suitable conceptual model to determine the solution for the problem. All the steps of the stimulus response model were studied in detail and it was modified using additional information gathered from literature. Marketing stimuli and environmental stimuli are the major external factors influencing consumer decision making and these stimuli enter into the consumer black box which contains consumer characteristics and buying process where its internal process is not seen to outsiders and the results of that internal process induce consumer to make own choices on products, brands, purchase timings and amounts (Kotler *et al.*, 2009). Accordingly, Figure 1 outlines the conceptual framework of this study.

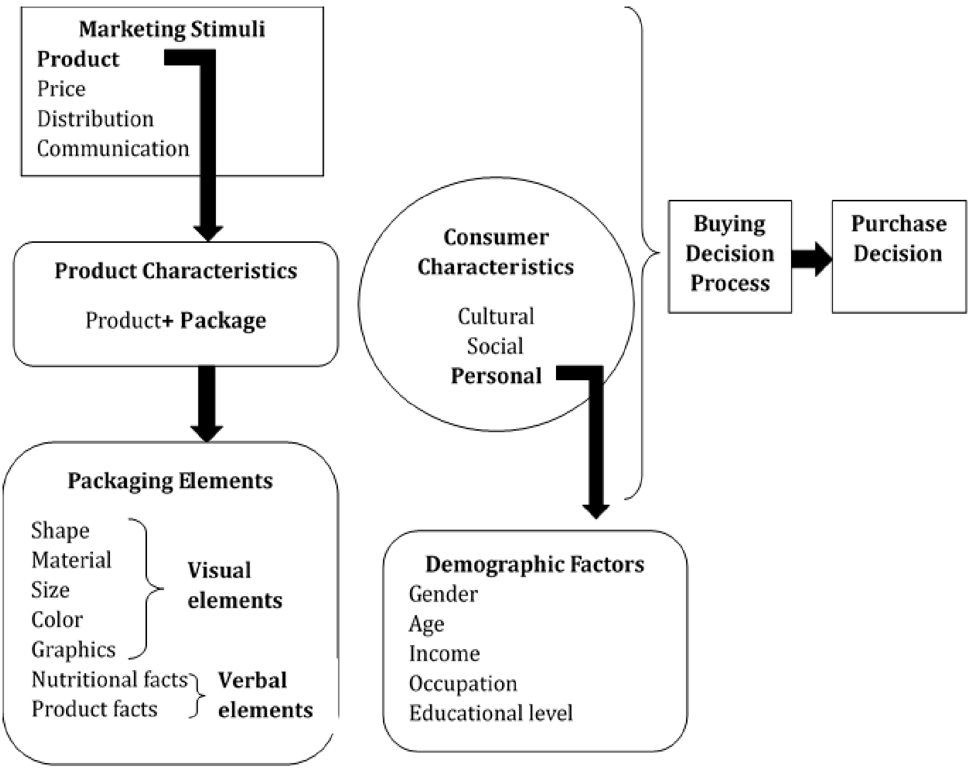

Figure 1: Conceptual framework of the study

The study mainly aims in identifying the impact of packaging elements on buying products with eco-friendly packages with a special reference to Sri Lankan dairy products including: fresh milk; flavored milk; powdered milk; curd; yogurt and cheese. Carton packages and glass bottles to pack fresh milks and flavored milks, cardboard boxes and glass bottles to pack powdered milk, clay pots to pack curd, clay containers to pack yoghurt and cardboard boxes and glass vessels to pack cheese products were considered as environmental friendly packaging material. The present study considered main two elements: visual and verbal in accordance with the past literature (Ghosh, 2016; Sultan, 2016). Visual elements including: shape; size; material; color and graphics were picked as the main variables for the study, while product and nutritional facts were considered as verbal elements.

Questionnaire items related to visual and verbal elements were taken as independent variables, while purchase behavior was taken as the dependent variable. All the measurements of the selected variables were taken using 5 point Likert scale. Cronbach's alpha values were calculated to confirm the reliability of the items used to measure the variables. Each item recorded more than 0.7 Cronbach's alpha value (Table 1) which verifies the higher reliability of the questionnaire (Taber, 2018). The questionnaire survey was carried out by distributing a pre-tested structured questionnaire among the consumers visiting Cargills Food city outlet located at Galgamuwa facing Kurunegala-Anuradhapura main road, Sri Lanka.

The sample was drawn from the consumers visiting Cargills food city outlet including 200 consumers who were selected randomly through time randomization during shopping hours. Data were collected within 10 consecutive dates of the month. When randomizing the time, the sales hours of the outlet extending from 10.00 am to 08.00 pm were divided into equal time slots where one day was divided into 40 equal 15-minute time slots. There were 400 (40×10) equal time slots available for the selected 10 consecutive days of the month. Out of 400-time slots, 200-time slots were randomly selected. The questionnaire survey was conducted by targeting the consumers who visit the outlet in the randomly selected 200-time duration of selected days.

Consumer buying behavior for selected dairy products: fresh/flavored milk; powdered milk; curd; yogurt and cheese was considered for the survey. Only 126 questionnaires were received out of the 200 questionnaires. However, only 104 completed questionnaires were used for the analysis. Thus, the final sample consisted of 104 consumers. Collected data were analyzed using statistical techniques including the Wilcoxon Signed-Rank test and Pearson's Chi-square test.

Table 01: Cronbach's Alpha values of questionnaire items

Statements	Cronbach's Alpha
1. Eco-friendly packaging purchasing behavior	
I often purchase dairy products with eco-friendly packaging	0.797
2. Nature of packing material	
Minimum materials should be used when designing eco-friendly packages	0.788
Reusable materials should be used when designing eco-friendly packages	0.779
Recyclable materials should be used when designing eco-friendly packages	0.775
Bio-degradable materials should be used when designing eco-friendly package	0.790
3. Visual elements of packaging	
Size influence the purchase decision of dairy products with eco-friendly packaging	0.773
Shape influence the purchase decision of dairy products with eco-friendly packaging	0.773
Material influence the purchase decision dairy products with eco-friendly packaging	0.794
Color influence the purchase decision of dairy products with eco-friendly packaging	0.780
Graphics influence the purchase decision of dairy products with eco-friendly packaging	0.778
4. Verbal elements of packaging	
Nutritional facts influence my purchasing decision of dairy products with eco-friendly packaging	0.773
Product facts influence my purchasing decision of dairy products with eco-friendly packaging	0.769

Results and Discussion

Demographic profile of the sample

Table 2 outlines the demographic profile of the selected sample.

Table 2: Demographic profile of consumers

Characteristic	Category	Frequency	Percentage (%)
Gender	Male	19	18.3
	Female	85	81.7
Age	10-20 years	2	1.9
	21-30 years	27	26
	31-40 years	24	23.1
	41-50 years	31	29.8
	Above 51 years	20	19.2
Income (Rs.)	Below 10,000	22	21.2
	11,000-20,000	14	13.5
	21,000-30,000	30	28.8
	31,000-40,000	30	28.8
	Above 41,000	8	7.7
Educational level	No schooling	2	2
	Grade 1-5	1	1
	Grade 6-11	17	16
	Up to Advanced level	53	51
	Higher education	31	30
Occupation	Government sector	24	23.1
	Private sector	33	31.7
	Self employed	14	13. 5
	Unemployed	33	31.7

Identifying the impact of visual elements of eco- friendly packaging on consumer purchasing behavior of dairy products

Wilcoxon Signed-Rank test was used to analyze the collected data to examine the relationship of packaging elements and consumer purchasing behavior of dairy products with eco-friendly packages. Shape, size, material, color and graphics of the product package were the independent variables considered for the test, and consumer buying behavior was the dependent variable. By considering the significant level <0.05, several visual elements of eco-friendly packages were highlighted as highly significant. From the results shown in Table 03, it is clear that package shape (p = 0.001), and package size (p = 0.001) were having a significant impact on buying dairy products with eco-friendly packages while package color (p = 0.706) was insignificant. Graphics of package (p = 0.064) proved to have a marginally significant impact (Dawar and Pillutla, 2000) on consumer buying behavior of eco-friendly packed dairy products.

Table 03: Results of Wilcoxon sign rank test on visual elements

Factor	Z value	P value
Shape	-9.048	0.001
Packing material	-7.412	0.001
Size	-5.029	0.001
Color	-0.377	0.706
Graphics	-1.849	0.064

Past literature proved that visual elements of product packages significantly influence consumer buying behavior (Alhamdi, 2020; Roy *et al*., 2021; Sung 2021). Further, Venter *et al*. (2011) expressed the comparable findings where, they argued that visual characteristics play a major role in gaining the attention of the consumers toward food packaging. Even though current research yielded that package color was insignificant in buying eco-friendly packed products, recent scholars showed contradictory evidence. They showed that, color is widely used as a cue to signal sustainability in the packaging designs of the products (Boz *et al*., 2020). For instance, green color is indirectly considered environmentally-friendly (Lindh *et al*., 2016). Numerous researches conducted in this field have highlighted packaging material as an essential factor for designing an eco-friendly package (Kuvykaite *et al*., 2009; Steenis *et al*., 2017; Zekiri and Hasani, 2015).

At the same time, the current study revealed that there is a significant association between consumer buying behavior and packing materials of dairy products particularly, eco-friendly materials; carton packages and glass bottles to pack fresh milks and flavored milks; cardboard boxes and glass bottles to pack powdered milk; clay pots to pack curd; clay containers to pack yoghurt and cardboard boxes and glass vessels to pack cheese products ($p = 0.001$). It implies that altering the suitable packing material of the dairy products will encourage the consumer to purchase them. However, Maleki *et al*. (2020) documented that packaging material does not encourage or discourage consumers to purchase a particular food product. The findings of the present study are of great significance for marketers to identify which packaging materials consumer values as the best material in packaging. Table 4 shows the results gained from the Wilcoxon sign rank test by analyzing the data collected on the nature of packaging material.

Table 04: Results of Wilcoxon sign rank test on nature of packaging material

Factor	Z value	P value
Minimally packed	-2.091	0.037
Reusability	-6.197	0.001
Recyclability	-7.412	0.001
Biodegradability	-5.600	0.001

By considering the significant level of <0.05, it is clear that there is a significant relationship between the minimally packed ($p = 0.037$) products or utilization of minimum materials when designing a package and buying behavior. Simply this means that consumers prefer purchasing products when the product is packed with a minimum amount of packaging material. This is in line with the findings of Haw and Xin (2021), who identified that light-weighting through the reduction in package material carries a positive impact on consumer purchasing behavior towards products with green packaging. With the optimum utilization of resources, the cost of production can be reduced when manufacturing at a large scale allowing more profits for the producer and less amount of waste disposal to the environment (Chan, 2000; Mendes and Pedersen, 2021).

Further, by considering the significant level of <0.05, the results revealed that utilization of reusable materials (p = 0.001), recyclable materials (p = 0.001), and biodegradable materials when designing a package (p = 0.001) have a highly significant impact on buying dairy products with eco-friendly packages (Table 4). This embellishes the findings of Orzan *et al*. (2018), who proved that recycling influences the decision to purchase ecologically packed products. The investigations conducted by Hao *et al*. (2019), similarly revealed that consumers attach a greater importance to the reusability of environmentally friendly packaging. The utilization of reusable packaging material has created a higher level of trust towards a product as it can be used for many years without dumping it into the environment (Lal *et al*., 2015). When designing a package for a product, glass, metals and aluminum can be used for manufacturing as the most common reusable materials.

However, opposite to the results of the present study, Martinho *et al*. (2015) have documented that there is no significant association between recycling and the buying behavior of products with sustainable packaging. As recyclable and biodegradable packaging materials are capable of arousing ecological cues in the consumer mindset, packages made out of them are more talented in attracting consumers (Magnier and Crie, 2008; Sharma *et al*., 2021). When designing a package biodegradable, recyclable packaging materials which are of high technology can be used for manufacturing packages.

Identifying the impact of verbal elements of packaging on consumer buying behavior of dairy products

As same as above, the Wilcoxon Signed-Rank test was used to analyze the consumer data to examine the relationship between the verbal elements of packaging and consumer purchasing behavior for dairy products with eco-friendly packages. Results of the Wilcoxon signed rank test obtained are depicted in Table 5.

Table 5: Results of Wilcoxon sign rank test on verbal elements

Factor	Z value	P value
Nutritional facts	-9.040	0.001
Product facts	-9.048	0.001

By considering the significant level of <0.05, the product facts (P = 0.001) and nutritional facts (P = 0.001) showed a significant impact on buying dairy products with eco-friendly packages (Table 5). This is in line with past researchers who have documented that the environmentally friendly nature of packaging was effectively communicated via verbal communications including claims and statements on product packages (Magnier and Schoormans, 2015; Pancer *et al*., 2017). Therefore, marketers should be well aware of what type of product details should be highlighted and included when designing a product package.

Relationship between demography of the consumer and buying dairy products with environmental friendly packages

It is vital to get an in-depth understanding of the relationship between the demographic variables and buying products with the environmentally friendly package. For that, Pearson's chi-square test was applied and Table 6 depicts the results.

Table 6: Results of the Pearson's Chi square test

Demographic Variable	X^2 value	Degree of Freedom(df)	P value
Gender	0.785	4	0.940
Age	3.998	4	0.406
Income	7.637	4	0.106
Education	12.339	4	0.015

Results of the current study revealed that gender (p = 0.940) is insignificant in buying products with environmentally friendly packages. This means that consumers buy products with environmentally friendly packaging despite their gender. However, contradictory results exist in past literature regarding the impact of gender on eco-friendly buying behavior. In that case, several scholars argued that females more often engage in eco-friendly buying behavior with a higher intention compared to males (Chekima *et al*., 2016; Kalamas *et al*., 2014; Matthes *et al*., 2014). In another study, it was found that, women aged above 50 showed a higher inclination for purchasing eco-friendly packed products (Martinho *et al*., 2015).

It is worth noting that results confirmed educational level significantly influences purchasing dairy products with an environmentally friendly package (= 0.015) while embellishing the past literature. Chekima *et al*. (2016) found out that the purchase intention of the consumers with higher education was enhanced by the eco-friendly attitude and eco-labels on the products. As postulated by Zhao *et al*. (2014), people who possess higher educational qualifications often have higher environmental knowledge and favorable environmental attitudes along with higher purchase intention toward green products in China. Being better informed, highly educated individuals possess a greater preference for environmental protection (Torgler and Garcia-Valinas, 2007). Interestingly, in the present study, occupation ($P = 0.064$) was found to have a marginally significant influence (Dawar and Pillutla, 2000) on consumer buying behavior with environmentally-friendly packages.

The present study proposes important managerial implications, particularly for marketers owing to the rising environmental and health concerns among Sri Lankan population. Accordingly, marketers should make efforts not only for producing eco-friendly packed products but also to formulate effective strategies and plans related to environmental-friendly packaging by taking into account the consumers' preferred elements of packaging. Since the educational level significantly influences the consumer buying behavior of eco-friendly packed products, marketers should focus on the packaging elements which effectively communicate the eco-friendliness of a product package. Furthermore, campaigns to raise the awareness level among consumers with less educational background will be beneficial to popularizing the purchase of eco-friendly packaged products.

Furthermore, marketers should try to incorporate consumer-preferred visual elements, for example: shape; size; packing material and graphics on the packaging when designing an eco-friendly package for a product while paying less attention to the package color. Managers must be well aware of the possible lower return on investment when investing in the color element on packages in the dairy products category in particular. Paying more attention to product and nutritional facts of packaging will be beneficial in attracting consumers towards eco-friendly packed products. Regarding the nature of packing material, consumers prefer minimum use of packing material usage in eco-friendly packages. Furthermore, manufacturers and marketers should design the packages using consumer-preferred packing materials which are reusable, biodegradable, and recyclable.

The theoretical part of the contribution was devoted to the elements of eco-friendly product packaging to gain a more competitive advantage for the business ventures. Most importantly, the influence of the package color on consumer buying behavior was insignificant out of the visual elements of packaging. Another essential point added to the existing knowledge is the significant relationship between consumers' occupation and buying behavior of eco-friendly packed products. In the Asian context, this study can be considered as one of the most comprehensive efforts in the direction of understanding the importance of visual and verbal elements, the nature of packaging material and demographic variables pertaining to dairy products with eco-friendly packaging as a whole. The addition of the Asian consumers' voice to the existing literature is another important theoretical contribution of the present study.

Conclusion

The present study aimed to identify the impact of eco-friendly packaging elements on consumer buying behavior. The study used the purchase of dairy products in particular because those are essential components of the human diet, consumed daily thus showing higher buying frequencies. The study found important findings related to the visual and verbal elements of eco-friendly packaging. Results revealed that visual elements such as package shape, material and size have a significant impact on the consumer purchasing behavior of eco-friendly packaged dairy products. Graphics on the package has a marginally significant influence on purchasing eco-friendly packed products. However, the package color has proven to have no significant impact on purchasing products with eco-friendly packaging. Verbal elements including product facts and nutritional facts had a significant influence on consumer purchasing behavior. Regarding the nature of packaging, minimally packed, reusable, recyclable and biodegradable product packages were significantly valued by consumers in the buying process. Furthermore, educational level and the occupation of the consumers have a significant influence on consumer `buying behavior with environmentally-friendly packages while gender, age and income are found insignificant.

Declaration of Conflict of Interest

Authors have no conflict of interest to declare.

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Growth, Physiology, Weed Abundance and Yield in Rice (*Oryza Sativa* L.) Under Three Nutrient Input Systems in the Dry Zone of Sri Lanka

P.D. Ariyasena[1], C. S. De Silva[2*], D.M.D. Dissanayaka[1], D.A.U.D. Devasinghe[1]

[1]*Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka.*
[2]*Faculty of Engineering Technology, Open University of Sri Lanka.*

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Abstract

A field experiment was conducted to compare the growth, physiology, weed abundance and yield in rice under three input systems; organic, conventional and integrated, during the 2019 *Yala* season as the second season of a long-term cropping systems trial. The design used a Randomized Complete Block Design (RCBD), with six (6) replicates for each treatment. The rice variety used in the experiment was Bg 300 and three treatments were based on three input systems such as T1 – Conventional input system (100% Department of Agriculture (DOA) inorganic fertilizer recommendation), T2 – Integrated system (50% of Department of Agriculture (DOA) inorganic fertilizer recommendation+50% of the rate of compost added to organic input system) and T3 – Organic system (No inorganic fertilizers and only compost with the rate of 10000 kg ha $^{-1}$). At the panicle initiation and harvesting stages, there was a ($p<0.05$) significant difference (in rice shoot biomass across the three input systems, with the organic system having the

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*Corresponding author:
Postal address: Faculty of Engineering Technology, Open University of Sri Lanka.
Email: csdes@ou.ac.lk

lowest values compared to the other two. At panicle initiation, 50 % heading, and harvesting phases, a significant difference ($p<0.05$) in plant height was noted among the three input systems, with the organic system showing the lowest values at all stages. The organic system had the highest weed count at all three crop stages and no significant difference between integrated and conventional systems. The three input systems had a significant difference in final grain yield at 14 percent moisture. At 14% moisture, there was no difference in final grain yield between conventional and reduced systems (4.85 and 4.67 tonnes/ha) with the organic system having the lowest value (2.74 tonnes/ha). Overall results indicate that reducing inorganic fertilizers by 50% with the combination of organic manure as in the integrated system gives similar final grain yields as the conventional system of new improved varieties like Bg 300 in high potential areas like Anuradhapura. Also, organic systems can show low crop growth and yields due to inadequate nutrient supply through organic materials and high weed density during the first year of transition.

Keywords: Crop growth; Physiological; Weed abundance; Organic; Rice

Introduction

In many parts of the world rice (*Oryza sativa* L.) is cultivated, and it is the staple food of many nations (Khairiah *et al*., 2009). Rice is known to be the single most significant crop occupying 0.9 million hectares in 2018 of the total cultivated area in Sri Lanka. In 2017/2018, an average of 560,000 and 310,000 hectares were farmed during *Maha* and *Yala*, with an average yearly extent of roughly 870,000 hectares sown. Around 1.8 million agricultural families are involved in cultivation around the island. Rice output in the country is currently 3.9 million tonnes per year, which meets around 95% of domestic demand (DOA, 2018). In Sri Lanka, rice yield is 4.30 tonnes/ha in 2018 (IRRI, 2019). After the green revolution, a challenge emerged as many farmers had to produce food for an ever-increasing world population using practices that increase crop production such as: the application of fertilizers and pesticides, intensive tillage and the use of monoculture (Benaragama *et al.,* 2016a). After the green revolution, a range of agricultural chemicals appeared in the sector all around the world. Chemical-based agriculture is rapidly becoming common also in the rice sector of Sri Lanka (Adhikari, 2011), mostly on Nitrogen, Phosphorous and Potassium.

Because of rapid response and visible benefits, most farmers tend to apply nitrogen to increase crop yield. As a result of maximising the use of inorganic fertilizers and pesticides, the use of improved high-yielding varieties that are receptive to these inputs was also increased. The government extension programs promote the use of inorganic fertilizers to increase yields and achieve self-sufficiency. Hence, inorganic inputs are a vital component of the rice sector of Sri Lanka.

Although inorganic inputs produce high yields, they cause significant environmental problem, including soil degradation, soil organic matter reduction, emission of greenhouse gases and adverse effects on natural ecosystems (Benaragama *et al*., 2016a). Problems associated with using excessive amounts of synthetic fertilizers and increasing the cost of these fertilizers are considered the major factors harmful to developing sustainable crop production systems (Adhikari, 2011). The dry zone is known as "the major rice growing area" in Sri Lanka, and rice cultivation is mostly carried out by conventional agricultural practices. In conventional agriculture practices, inorganic inputs are applied to the crop to maintain productivity. The use of excessive amounts of synthetic fertilizers, as well as the rising expense of these fertilizers, is recognized to be hazardous to agricultural production systems' sustainability (Adhikari, 2011).

Over the last 15 years, Chronic Kidney Disease (CKD) has gradually developed, impacting the low socioeconomic agricultural population in the North central province and it is thought to be attributable to the increasing usage of inorganic fertilizers. Also, farmers have to disburse a lot of money for fertilizers and other chemicals. This resulted in environmental pollution, the development of pest and disease resistance and an increase of the cost of production (Nanayakkara and Upul, 2012). Alternatively, organic crop production systems are gaining popularity. Therefore, this research was conducted to promote organic and integrated nutrient management systems to overcome problems that occurred due to conventional farming. The main goals of this study are: to compare plant growth, physiological and yield parameters in organic, integrated and conventional input systems as well as to figure out how these different input systems affect weed abundance and competition in rice during the *Yala* season in the first year of transition.

Methodology

Experimental location

A field trial was carried out during the 2019 *Yala* season at the Research Unit of the Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura district (located in 8° 22'14.84" N and 80° 25'13.66" E). This area belongs to the agro-ecological zone of the low country Dry Zone (DL1b) (Thenabadu, 1988). The mean annual rainfall and the average temperature of the area vary from 1250 to1750 mm and 25 to 30 respectively. The study area consists of imperfectly drained Reddish-Brown Earth soils (Soil taxonomic Order - Alfisols, Suborder - Ustalfs, Great Group - Haplustalfs) (Mapa *et al*., 2010).

Experimental design and treatments

The experimental design was RCBD and there were six (6) replicates for each treatment combination. The tested rice variety was Bg 300. Three treatments were based on three input systems as T1 – Conventional input system (100% N supplied by an inorganic fertilizer application based on the recommendation of the Department of Agriculture), T2 – Integrated system (Reducing the inorganic and organic fertilizer system by 50%) and T3 – Organic system – 50% N supplied by organic fertilizers (The Nitrogen content of organic fertilizer was previously calculated to determine the relevant rate to get the required Nitrogen content).

Plot preparation, crop establishment and management

Plots of 15 m × 6 m dimensions were made. Altogether 6 plots were prepared for one treatment and therefore, 540 m^2 were achieved as the total plot area per treatment. Land preparation of rice was carried out as per the lowland rice cultivation method. Each plot was submerged and soaked for 3 days. Then, the land was ploughed with a power tiller and impounded with water for two weeks before the establishment of the crop. After one week of the first ploughing, the second ploughing was done crosswise with the power tiller. Then bunds were reshaped. Before the establishment, the cropland was puddled and levelled. On the puddled and levelled plots, pre-germinated seeds of the Bg 300 rice variety were dispersed at a rate of 120 kg ha^{-1}.

Fertilizer application

The input systems were defined based on the elemental N supply and the sources. In organic system, the Phosphorus and Potassium rates were not standardized. The amounts of these two elements were dependent on the quality of materials used to supply N. Cow dung and compost were the organic nutrient sources used.

Table 1: Fertilizer rates added to conventional and integrated systems by mineral nutrients and alternative sources

Input system	Mineral nutrient (kg/ha)	Nutrients from alternative sources (kg/ha)
Conventional	N – 103.5 (Urea 46%) P – 3.9 (P_2O_5 43.7%) K – 30.0 (K_2O 60%)	N – 0 P – 0 K – 0
Integrated	N – 51.8 (Urea 46%) P – 1.9 (P_2O_5 43.7%) K – 15.0 (K_2O 60%)	N – 25.9 P – 0.65 K – 52.5

Weeds, insects, pest and disease management

Weed management of conventional and integrated plots was carried out by applying synthetic herbicide Sofit (Pretilachlor+safener) which is a pre-emergence herbicide. MCPA (2-methyl-4-chlorophenoxyacetic acid) was added 20 DAS. Carbosulfan 200 g L^{-1} SC was added to the field to control pests of paddy bugs, brown plant hoppers and thrips to the conventional and to the integrated system. Neemzal (neem (*Azadirecta indica* L.) based commercial organic pesticide) was applied to the organic system.

Agronomic practices and irrigation

All agronomic practices were carried out following the Department of Agriculture recommendations in Sri Lanka. Irrigation was started one week after the crop establishment and impounded with a 5 cm depth of water to keep the soil sufficient moist throughout the experimental period. The flood irrigation method was followed to keep the soil moist.

Data collection

Plant growth was measured in 20 days of intervals in seedling, panicle initiation, 50% heading and harvesting stages. Physiological parameters were taken at panicle initiation and 50% heading stage. Grain yield was measured at crop maturity after 90 days after establishment (harvesting stage). Weeds were counted at seedling, 50% heading and maturity stages. Biomass of weeds was measured at 50% heading and maturity stages.

Measurement of plant growth parameters

For measuring plant growth parameters destructive sampling techniques were practiced at seedling, panicle initiation, 50% heading and harvesting stages. The sampling was done using a quadrate of the size of 50 × 50 cm. Quadrate was placed in four places on each plot and the plant samples were collected, for most of the parameters. When it deviates from normal practice, it was highlighted in appropriate places.

Plant density

At the seedling and harvesting stages, the number of plants per unit area was measured. All the plants collected from the quadrate in four different places in a plot were counted to measure the number of plants in m^2 for each plot.

Average plant height

Randomly selected 12 seedlings per quadrate were taken to measure this parameter. At two stages (Seedling and panicle initiation), the height from the base of the plant to the tip of the fully developed leaf (in cm) was measured. At the 50 percent heading stage and maturity stage, the height from the base of the plant to the tip of the panicle was measured in cm.

Number of tillers

The number of tillers was counted by using randomly selected 12 plants that were used for the height measurements. From panicle commencement to harvesting, the number of tillers per plant was counted.

Crop biomass (shoot dry weight)

After taking measurements of plant height and tiller count, the same plants were taken to measure shoot dry weight. Plants were uprooted and thoroughly cleaned to remove any undesired contaminants, after which they were air dried. Then, all 12 plants from each plot were put separately into paper bags and then was oven dried at 60 °C until getting a constant weight and total dry weight measured per plant basis at the seedling to harvesting stage (Wijayawardhana *et al*., 2016).

Measurement of plant physiological parameters
Relative leaf chlorophyll content

At the panicle initiation and 50% flowering stages, relative leaf chlorophyll content was evaluated as a SPAD (Soil Plant Analysis Development) value using a SPAD-502- leaf chlorophyll meter. Four plants in four different places from a plot were selected to measure this parameter. Then three fully expanded leaves that arose from the top of the plant were used for readings. The SPAD values in three different places in one leaf were measured and the average of them was taken as the leaf relative chlorophyll value.

Photosynthetic rate, stomatal conductance to water vapour and leaf temperature

These parameters were measured using a portable photosynthetic meter at panicle initiation and 50% flowering stages.

Light interception

Light interceptions of the crop field were measured using Line Quantum Sensor (LI-COR LI-191R) when the field received uniform solar radiation at 50% heading stage. Here, two locations in a plot were selected considering the healthy and uniform growth of the crop in the plot. Then, the LI 250A Light meter value at the eye level of the operator was taken by levelling the instrument horizontal to the ground and recorded as the top value of the incoming, reflected, and transmitted Photosynthetically Active Radiation (PAR). Later, the instrument was placed under the crop canopy, just above the ground level in two positions by making a cross in the position. Then, PAR was measured at the position and was recorded and the average of the two values was taken as the bottom reading of the light meter. Finally, the difference between the top reading and the bottom reading was taken as the light interception value.

Electrolyte Leakage (EL)

Healthy grown two plants from a single plot were selected to measure EL. Flag leaf from each plant was detached from the rice plant at the pre-dawn stage of the day and the fresh weight of the leaf was taken (W). Then, the leaf was cut into 1 cm length pieces. The leaf pieces were washed in deionized water, blot dried on tissue papers and placed in 20 mL of deionized water containing polypropylene tubes at room temperature for 1 hour with gentle shaking. Then, the Electrical Conductivity (EC) of the bathing solution (EC1) was measured using an EC meter. The total EL was measured after freezing subsequent samples at –60 °C for 12 hours (EC2). Then EL was expressed in per gram of leaf tissue using the following equation.

Measurement of weed parameters
Weed density

The quadrate was used to count the number of weeds per unit area at the seedling stage, rather than uprooting the plants (Nkoa *et al.,* 2015). Weeds from four different locations of a single plot was selected and uprooted using the quadrate as explained in the Measurement of plant growth parameters section and counted according to categories of broad leaves, sedges and grasses at the 50% heading and harvesting stages.

Weed biomass

At 50 percent heading and harvesting, weed dry weight was measured. All of the weeds that were gathered to determine weed density per plot were rinsed thoroughly with tap water to remove any undesirable soil. The weeds were then air dried for two days before being oven dried to achieve constant weight, similar to how shoot biomass was measured in the crop biomass part.

Measurement of yield parameters

To measure the yield parameters, the first plant samples were collected using a quadrate as explained in the measurement of plant growth parameters section. Then all the panicles were separated from the plants. After that, the panicle was grouped according to the height of the panicles as high, medium and low per plot. Then 20% of the panicles were selected from each group to measure all other yield components per each plot.

Number of spikelet/panicle

Separated panicles were counted per plot. Then, the number of grains in separated panicles was counted per plot. Finally, the grain count for separated panicles was divided by separated panicle count to measure the number of spikelets/panicle per plot.

Number of filled spikelets/panicle

Grains were placed into a water basin and, grains floated were considered as unfilled grains and separated. Then the number of filled grains was counted for separated panicles and, divided by separated panicle count to measure the number of filled spikelets/panicle per plot.

Final grain yield at 14% moisture

Grain yield was corrected to 14% moisture. Final grain yield was calculated for a square meter (m^2) and then converted into tonnes/ha per each plot.

Harvest index

The percentage ratio of grain yield to grain plus straw yield of each plot was used to determine the Harvest index.

Data analysis

All the parameters were analysed using ANOVA Mixed procedure in SAS. Means were separated using LSD mean at p = 0.0

Results and Discussion

Plant growth parameters

Plant density

A significant difference was observed in plant density among the three input systems at the seedling stage. However, at the harvesting stage, no difference was observed among the three input systems (Table 2). At the seedling stage, the maximum plant density was identified in the integrated input system and the lowest was in the conventional system and the organic system showed an intermediate value. Yield does not depend solely upon the performance of the individual plants, but also on the total number of plants per plot and yield components within a plant. Wider spacing had a linear effect on boosting the performance of individual plants, according to Baloch *et al*, (2002). Increased spacing allows plants to draw more nutrients from the land around them and absorb more solar radiation for a better photosynthetic process, resulting in improved individual plant performance (Baloch *et al*., 2002). Number of tillers

There was a significant difference in tiller count among the three input systems only at 50% heading stage (Table 2). The number of tillers has been detailed to have a positive affiliation with plant biomass and financial yields in rice (Deng *et al*., 2015). At the panicle initiation stage, no differences were observed in tiller count among treatments. At the 50% heading stage, the conventional system had the maximum tiller count, and no differences were observed between integrated and organic systems. At the harvesting stage, the conventional system had the maximum tiller count and the organic system had the lowest. At all three stages, the organic system had the lowest tiller count due to P and N deficiency. As a result of Phosphorus deficiency reduced shoot growth and lesser tiller production in rice plants can be observed (Luquet *et al*., 2005).

A study by Kekulandara *et al* (2017) also reported that, there was a noteworthy delay in tillering in a Phosphorus lacking condition. The application of N fertilizer is the foremost common and viable way to enhance the tiller populace because, it increments the *cytokinin* substance inside tiller hubs and, assist improves the germination of the tiller primordium (Liu *et al.,* 2011). Similar studies that have measured morpho-physiological parameters of rice genotypes (Epstein and Bloom, 2005; Islama *et al*., 2009) also showed increase of tiller count with increase of N content.

Shoot biomass

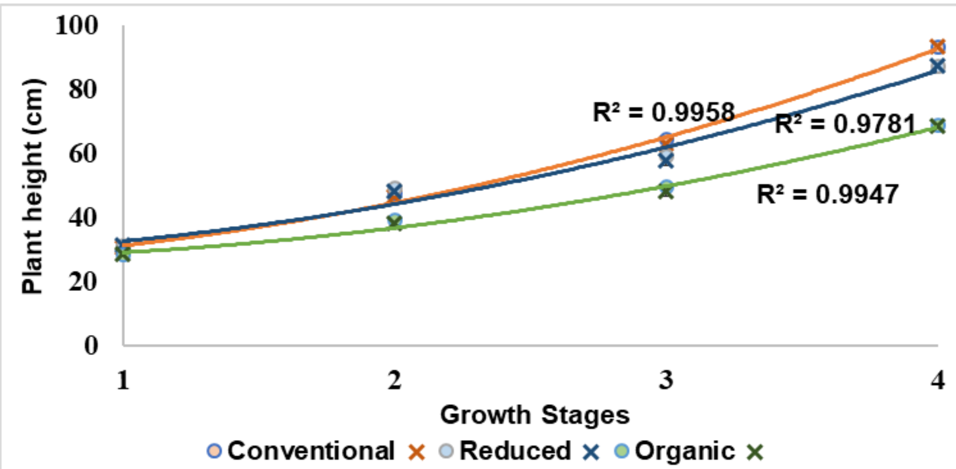
A significant difference was observed in shoot biomass among the three input systems at panicle initiation, 50% heading and harvesting stages. Mean shoot dry matters of rice in different growth stages among three input systems are presented in Table 2. At the seedling stage, no differences were observed in shoot biomass among three input systems because, at the initial stage plants do not absorb many nutrients from the soil but, depends on the nutrients in the seed. At panicle initiation, 50% heading and harvesting stages, no differences were observed among conventional and integrated systems due to the continuous application of inorganic fertilizers and their availability, and organic system had the lowest shoot biomass. Significantly low shoot biomass recorded in organic system in panicle initiation, 50% heading and harvesting stages indicate lack of soil fertility. The shoot biomass was significantly affected by major nutrients as N, P and K and also Zn (Nawaz *et al*., 2015). Also, *Fageria* and *Filho* (2001) and *Fageria* and *Baligar* (2005) have stated the increment in plant height and shoot dry weight with increasing nitrogen rate.

Table 2: Effect of three input systems on Plant growth parameters (plant density, tiller count and shoot biomass)

	Plant density (Plants/m^2)	**Tiller count/plant**	**Shoot biomass(g)**					
	Harvest stage (90DAS)	**Panicle initiation stage (40DAS)**	**50% heading stage (60DAS)**	**Harvest stage (90DAS)**	**Seed ling Stage (20DAS)**	**Panicle initiation stage (40DAS)**	**50% heading stage (60DAS)**	**Harvest stage (90DAS)**
Conventional	181.98^{a}	1.53^{a}	1.52^{a}	1.3^{a}	1.37^{a}	9.06^{a}	12.70^{a}	19.55^{a}
Integrated	244.69^{a}	1.53^{a}	1.22^{b}	1.18^{ab}	1.51^{a}	9.60^{a}	11.23^{ab}	16.09^{a}
Organic	240.52^{a}	1.43^{a}	1.19^{b}	1.07^{b}	1.24^{a}	6.07^{b}	9.70^{b}	9.53^{b}
Standard Error	24.78	0.07	0.12	0.07	0.09	0.69	0.67	1.35
Significant (P-value)	0.21	0.47	0.02	0.1	0.15	0.01	0.02	0.0004

Plant height

A significant difference was observed in plant height among three input systems at panicle initiation, 50% heading and harvesting stages (Figure. 1). At panicle initiation stage, no differences were observed in plant height among conventional and integrated systems and, organic system had the lowest value. Conventional and integrated systems had greater plant height compared to the organic system probably due to high level of major nutrients (N, P and K) (Iqbal *et al*., 2017). At both 50% heading and harvesting stages, the three systems showed differences in plant height, where the highest in the conventional followed by integrated and organic systems, respectively. Synthetic fertilizer provides nutrients which are readily soluble in soil solution and thereby instantly available to plant. Islam *et al*., 2017 mentioned that plant height has significant positive association with rice grain yield.



1 – Seedling stage; 2 – Panicle initiation stage; 3 – 50% heading stage; 4 – Harvesting stage

Figure 1: Differences in plant height among three input systems at seedling, panicle initiation, 50% heading and harvesting stages.

Physiological parameters
Chlorophyll content (SPAD Value)

A significant difference was observed in leaf Chlorophyll content among three input systems at panicle initiation and 50% heading stages. At both panicle initiation and 50% heading stages, the conventional system had the highest relative Chlorophyll content and organic system had the lowest (Table 3). Leaf Chlorophyll content is dependent on the Nitrogen content and the colour of the leaf; however, this correlation varies with plant growth stage and/or variety mostly because thickness of the leaf or leaf weight (Dissanayake *et al*., 2014). Chlorophyll content was significantly low in the organic input system in both growth stages when compared to conventional and integrated systems due to applying Nitrogen through inorganic fertilizer for both integrated and conventional systems and not for the organic system. Also, past research (Swain and Sandip, 2010) found that the SPAD value was linearly correlated with leaf Nitrogen content and Varvel *et al*. (1997) demonstrated N fertilizer significantly increased SPAD reading.

Electrolyte leakage

Differences in the electrolyte leakage (EL) have been known to detect stress injury of cell membrane. EL varies in relation to the ability of membrane to take up and retain solutes and, therefore, reflects stress induced changes in both membrane potentials and membrane permeability (Agarie *et al*., 1995). There was a significant difference in EL among three input systems at panicle initiation stage. Organic input system showed the highest EL, and no differences were observed among conventional and integrated systems (Table 3).

Deficiency of nutrients and environment stress conditions may be the reason for plasma membrane damage and increase of EL of organic system. Salt stressis also a reason for gradual increase of EL causing damage to the membrane (Fazal and Bano, 2016). To overcome the negative effect of salinity, the addition of different source of Nitrogen and Potassium to growth media as an ameliorative agent could be essential.

Table 3a: Effect of three input systems on Plant physiological parameters (leaf chlorophyll content (SPAD value), stomatal conductance to water vapor, leaf temperature).

	Leaf chlorophyll content (SPAD value)		**Stomatal conductance to water vapor (*u*mol/m^2/s)**		**Leaf temperature (^{o}C)**	
	Panicle initiation stage (40DAS)	**50% heading stage (60DAS)**	**Panicle initiation stage (40DAS)**	**50% heading stage (60DAS)**	**Panicle initiation stage (40DAS)**	**50% heading stage (60DAS)**
Conventional	36.29[a]	36.24[a]	-0.04[a]	0.41[a]	28.55[a]	28.29[c]
Integrated	31.21[b]	32.84[b]	0.05[a]	0.42[a]	28.46[a]	30.25[a]
Organic	25.84[c]	26.98[c]	-0.007[a]	0.24[a]	28.29[a]	29.57[b]
Standard Error	1.03	1.40	0.13	0.06	0.23	0.16
Significant (P value)	<.0001	<.0001	0.67	0.11	0.72	<.0001

Photosynthetic rate

There was a significant difference in photosynthesis among three input systems at panicle initiation and 50% heading stages. At both panicle initiation and 50% heading stages, conventional system had the highest photosynthetic rate. At both stages, no differences were observed among organic and integrated input systems (Table 3). It is fact, that P and N are contributed on regulating various photosynthetic processes and also that N: P ratio is essential for plant growth. As a result of Nitrogen and/or Phosphorus deficiency, a reduction in the photosynthetic capacity will emerge which can mainly be affect the dysfunction of the Calvin cycle and will limit nutrient supply to the chloroplasts (Conversa *et al*., 2015) and this could be the reason for low photosynthesis recorded at both panicle initiation and 50% heading stages in organic system because its wasn't treated with inorganic fertilizers containing N and P. Conventional system showed the highest photosynthetic values at both stages and integrated system showed comparatively a low value compared to conventional system because for integrated system, inorganic and organic manure were reduced by 50%, and therefore, N and P content are reduced. Amount of Chlorophyll are closely related to the photosynthetic rate. When the amount of Chlorophyll is high in the leaves, a higher photosynthetic rate can also be identified (Hidayati *et al*., 2016).

Leaf temperature

At panicle initiation stage, no significant difference was observed in leaf temperature among three input systems. A significant difference was identified in leaf temperature among the three input systems at 50% heading stage and integrated system had the highest leaf temperature and conventional system had the lowest. The low leaf temperature decreases the use of breath and helps keeping normal physiological functions of leaves (Yun-Ying *et al*., 2009). Rice varieties with lower leaf temperatures are thought to have higher photosynthetic and transpiration rates while also producing high yields (Hirayama *et al*., 2006). The above statements justify the results of this study by showing that, at 50% heading stage, conventional system had the lowest leaf temperature (28.28 ˚C) and the highest photosynthesis value was also recorded in conventional system. Idso *et al*. (1977) reported similar findings for wheat, indicating that leaf temperature during the heading stage was closely connected (Positive relationship) to grain yield and could be used to estimate yield in wheat varieties.

Light interception

There was a significant difference in Light Interception (LI) among the three input systems at 50% heading stage. LI at 50% heading stage, conventional and integrated system showed statistically similar mean values (93.02 and 97.13 µmol/ m^2/s, respectively) and organic system had a lowest value compared to conventional and integrated systems (57.14) as shown in Table 3. It is because, compared to the plants in the organic system, plant growth and tillering capacity was high in conventional and integrated systems causing greater LI. The Leaf Area Index (LAI), plant population, spatial organization, and, most importantly, canopy structures all play a role in LI by a crop canopy. Nitrogen stimulates the production and retention of leaves.

Therefore, LI would be greater in treatments receiving more applied N (Weerakoon *et al*., 2000). This supports the findings of this research as conventional and integrated systems received more applied N and therefore, high values of LI than the organic system which with low N levels. Similar observations were made by Kumar and Gangwar (1985) and Haque *et al*., (2006) by showing LI would be greater in treatments receiving more applied N.

Stomatal conductance to water vapour

There was no significant difference in stomatal conductance to water vapour among three input systems at panicle initiation and 50% heading stages. Rice grain yield was found to be highly associated with leaf photosynthetic rate and stomatal conductance in recent research (Takai *et al.,* 2009).

Table 3b: Effect of three input systems on Plant physiological parameters (photosynthetic rate, light interception and electrolyte leakage).

	Photosynthesis rate ($\mu mol\ CO_2/m^2/s$)	**Light interception ($\mu mol/\ m^2/s$)**		**Electrolyte leakage**
	Panicle initiation stage (40DAS)	**50% heading stage (60DAS)**	**50% heading stage (60DAS)**	**Panicle initiation stage (40DAS)**
Conventional	24.91[a]	14.44[a]	93.02[a]	0.76[b]
Integrated	19.07[b]	11.18[b]	97.13[a]	0.97[b]
Organic	12.90[c]	7.49[c]	57.14[b]	7.34[a]
Standard Error	2.40	1.28	8.47	0.74
Significant (P value)	0.0003	0.0002	0.02	<.0001

Because H_2O and CO_2, which are involved in the photosynthetic process, must pass through the stomata before entering mesophyll cells and chloroplast stroma, stomatal conductance is important in generating photosynthesis in rice plants (Doni *et al.,* 2014). Several studies have reported a close relationship between stomatal conductance and photosynthetic rate (Lin *et al*., 2005). However, in this study such relationship was not observed

Weed abundance

Weed density

Weeds compete with rice for light, nutrients, water, and other development requirements due to their fast growth rate, ability to adapt to changing conditions, and more efficient seed production. Environmental and edaphic conditions favorable for growing rice are also favorable for growing and reproducing many terrestrial, aquatic and semiaquatic weeds (Smith, 1988; Kim and Moody, 1989). Grasses are usually the most dominating rivals in the early phases of rice production, whereas sedges and broad-leaved weeds take over in the later stages (Jiang, 1989). A significant difference in weed density was observed among the three input systems at seedling, 50% heading and harvesting stages. At all three stages, organic system had the highest weed density, and no significant difference was observed among integrated and conventional systems (Table 4). It is because there was no application of pre-emergent herbicide to the organic system, but it was applied to both integrated and conventional systems. This supports a prior study of Benaragama *et al*. (2016a) which showed greater weed density in organic systems with the exclusion of synthetic pesticide/fertilizer and genetically modified inputs, when compared to conventional systems.

Table 4: Effect of three input systems on weed density

	Weed density (Number of weeds /m^2)		
	Seedling Stage (20DAS)	**50% heading stage (60DAS)**	**Harvesting stage (90DAS)**
Conventional	160.83[b]	14.67[b]	6.83[b]
Integrated	236.83[b]	30.83[b]	39.17[b]
Organic	2225[a]	158.33[a]	536.33[a]
Standard Error	136.78	6.59	45.81
Significant (P value)	<.0001	<.0001	<.0001

Weed biomass

At both 50% heading and harvesting stages, weed biomass among three input systems was significantly different (Table 5). Organic system had the largest weed biomass, integrated system had the lowest and conventional system had an intermediate value at 50 percent heading stage. However, no differences were observed in weed biomass among conventional and integrated systems at harvesting stage. However, organic system resulted the highest weed biomass probably due to the application of herbicides to conventional and integrated system and not for the organic system. At this point, the organic system had four times the weed biomass of the conventional system, which was consistent with the findings of many others (Benragama *et al*., 2016b; Davis *et al*., 2005; Ryan *et al*., 2009). However, treatments with high weed density did not necessarily have high weed biomass, indicating that there is no link between weed densities and weed biomass. When compared to conventional and integrated systems, organic systems exhibited a higher weed biomass at harvest, which could be one of the reasons for the organic system's lower crop production.

Table 5: Effect of three input systems on weed biomass

	Weed biomass (g)	
	50% Heading stage (60DAS)	**Harvesting stage (90DAS)**
Conventional	5.12[ab]	6.28[b]
Integrated	2.82[b]	5.50[b]
Organic	6.09[a]	24.14[a]
Standard Error	0.84	3.46
Significant (P value)	0.04	0.0002

Yield components

Number of spikelet/panicle

The number of spikelets/panicles varied significantly among the three input systems (Table 6). Conventional input system had the highest number of spikelet/panicle and organic system had the lowest. Three yield components, panicles per plant, grain weight, and spikelet number per panicle were used to calculate rice grain yield (Tian *et al*., 2006). As a result, the following statement can be used to validate the findings of this study because the conventional method produced the maximum number of grains per panicle, as well as the highest yield. In terms of final yield values, the integrated system had the second highest value of spikelet per panicle, while the organic system had the lowest. As can be seen, the number of spikelets per panicle is an important yield component that impacts the ultimate yield.

Number of filled spikelet/panicle

The amount of filled spikelets per panicle varied significantly between the three input systems. Conventional system had the highest value, organic system had the lowest value and integrated system had a value in between them (Table 6). Drought, low solar radiation, Nitrogen deficit, low or high temperatures and panicle blast can all increase the amount of empty spikelets and, as a result, grain output. Rice genes also control spikelet sterility and empty grains. (Fageria, 2007). Nitrogen deficiency could be the main reason for low number of filled grains in organic system (35.9) and then, higher values 70.5 and 50.6 filled grains per panicle were for conventional and integrated systems respectively, because N nutrients were adequately supplied from inorganic fertilizers to those two systems. Dissanayake *et al*., 2014 reported comparable findings for Bg 352 rice variety with identical fertilizer combination in *Maha* and *Yala* seasons.

Final grain yield at 14% moisture

The three input systems had a significant difference in final grain yield at 14 percent moisture. At 14% moisture, there was no difference in final grain yield between conventional and reduced systems, with the organic system having the lowest value (Table 6). This could be due to the contribution of chemical fertilizers in reduced and conventional systems, as well as the higher weed density in organic systems compared to reduced and conventional systems, both of which positively influenced all of the rice plant's yield contributing characters as observed in this study. The organic system's lack of soil fertility was reflected in plant growth parameters and, ultimately, crop grain production. Weed-related yield losses have been calculated in various rice-producing countries. Losses in India have been estimated at 10% of the crop. Losses in the Philippines were predicted to be 11% for the dry season and 13% for the wet season (Datta, 1981). However, according to a previous study (Benaragama *et al.*, 2016b), organic management practices and/or greater crop rotation diversification did not improve yield or minimize yield loss due to weed competition because of factors linked with poorer soil fertility. In this study, results show that reducing agrochemicals (fertilizer and herbicides) as was done in the reduced system does not affect the final grain yields, and similar results were shown in prior studies by (Benaragama *et al.,* 2016a) and (Dissanayake *et al*., 2014).

Harvest index

Harvest index is defined as the ratio of grain yield to total above-ground biomass (straw and grain) and is used to determine biological success in partitioning absorbed photosynthate to the harvestable product or simply how efficient a crop is at producing grains. (Li *et al*., 2012; Dissanayake *et al*., 2014). There is a significant difference in harvest index among the three input systems (Table 6). Conventional system had the highest harvest index and there was not any difference observed among integrated and organic systems. The number of panicles per unit area, the number of spikelets/panicle, the weight of 1,000 mature kernels and the percentage of fully ripened grains all contribute to the harvest index of rice (Li *et al*., 2012). Although the number of panicles in the integrated system is the largest of the three treatments, the number of grains per panicle and the number of filled grains per panicle are lower than in the conventional system, which is likely why the integrated system has a low harvest index. For rice, published harvest index values range from 0.35 to 0.55 (Fageria *et al*., 2003). In contrast, harvest index ranged between 0.39 to 0.46 because variety Bg 300 is a new improved rice variety. Dissanayake *et al*., 2014 found similar findings for the Bg 352 rice variety in conventional, integrated and organic systems, with harvest indexes ranging from 0.40 to 0.46.

Table 6: Effect of three input systems on yield parameters

	Number of spikelets/panicle	Number of filled spikelets/panicle	Final grain yield at 14% moisture (tonnes/ha)	Harvest index
Conventional	87.45[a]	70.47[a]	4.8519[a]	0.4666[a]
Integrated	60.63[b]	50.63[b]	4.6661[a]	0.3985[b]
Organic	42.09[c]	35.85[c]	2.7393[b]	0.4056[b]
Standard Error	0.2765	0.2752	0.3944	0.0174
Significant (P-value)	<.0001	0.0001	0.0006	0.0294

Conclusions

During the first year of transition, this study compared rice growth, physiology, weed abundance and yield using three distinct input systems (conventional, integrated and organic). Plant physiological parameters such as leaf Chlorophyll content and photosynthesis rate were nearly identical and higher in conventional and integrated systems than in organic systems at all stages, owing to adequate nutrient supply provided by inorganic fertilizers in conventional and integrated systems. At seedling stage, crop growth was not shown many differences among the input systems indicating less reliance on soil fertility but rely upon the nutrients stored inside the seeds for growth. However, after seedling stage, there was a larger rate of variances in growth metrics, with conventional and integrated systems showing more plant growth than the organic system.

When compared to conventional and integrated systems, the organic system's lack of weed control resulted in high weed density and biomass at all stages. Overall results indicate that reducing inorganic fertilizers by 50% with the combination of organic manure as in the integrated system gives similar grain yields as the conventional system for a new improved variety like Bg 300 in high potential area like Anuradhapura. Also, organic systems can show low crop growth and yields due to inadequate nutrient supply through organic materials and due to high weed density during the first year of transition. Therefore, further studies are needed for the determination of a sustainable organic material that can adequately supply the nutrient requirement and exact quantities of them. Moreover, further studies are needed to identify different problems occurred in organic rice cultivation and to ensure the sustainability of integrated (reduced) nutrient management systems.

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Paddy Yield Estimation Models Using Satellite Data: A Case Study in Kamburupitiya DS Division

P.K.R.N. Gunapala[1*], C.P. Rupasinghe[1] and A.C.P. Priyankara[2]

[1]*Department of Agricultural Engineering, Faculty of Agriculture, University of Ruhuna, Sri Lanka*
[2] *Department of Information Technology, Faculty of Agriculture, University of Ruhuna, Sri Lanka*

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Abstract

In Sri Lanka, the crop cutting survey method is used to estimate the average paddy yield during the harvesting period, and it has failed to forecast the yield prior to harvesting. This method is time-consuming, expensive and make various errors. The yield prediction is worth to estimate yield before harvesting thousand tonnes of paddy. Satellite Remote Sensing is used to forecast crop yield using images during vegetative growth. The research was conducted in Kamburupitiya Divisional Secretariat (DS) division, Matara District, Sri Lanka, during *Yala* and *Maha* paddy growing seasons. Landsat 8 OLI/TIRS images with 30 m resolution were utilized to develop the paddy yield models using paddy yield records during 2016 to 2020. The simple linear regression models were developed using recorded average paddy yield data of the Kamburupitiya DS division and the average values of respective vegetation indices (Normalized Difference Vegetation Index (NDVI); Green Vegetation Index (GVI); Difference

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*Corresponding author
Postal address: Department of Soil Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka.
Email: ruwanthikanishani3@gmail.com

Vegetation Index (DVI); Infrared Percentage Vegetation Index (IPVI) and Ratio Vegetation Index (RVI) and reflectance values of three spectral bands (Green (G), Red (R) and near-infrared (NIR)). The validation process was carried out using Mean Absolute Percentage Error (MAPE) as the statistical indicator between modeled and predicted yield. When considering coefficient of determination (R^2) value, MAPE percentage, Durbin Watson statistics values, and Pearson correlation values, the yield model based on the GVI for *Maha* season and NDVI based model for *Yala* season are selected as the best models for paddy yield prediction using Landsat 8 OLI/TIRS imagery. The models will allow authorities to make effective decisions in paddy supply management.

Keywords: Paddy; Remote sensing; Spectral bands; Vegetative index; Yield prediction

Introduction

Rice is a major staple food crop that occupies 34% of the total cultivated lands in Sri Lanka (Department of Agriculture, 2020). Paddy is cultivated in Sri Lanka as a wetland crop in two major growing seasons, *Yala* and *Maha* (Census and Statistics, 2020). Generally, the "crop cutting survey" method estimates the average paddy yield during the harvesting period in Sri Lanka. This method was introduced by the Food and Agriculture Organization of the United Nations (FAO) in 1951(Census and Statistics, 2020). This is done using ground collected data (Census and Statistics, 2020), but crop cutting survey method is time-consuming, expensive, and has the potential to make various errors. Rather than that, it fails to predict the yield before harvesting. Due to unpredictable yield variations, food insecurity and management problems: scarcities and surpluses of paddy yield can happen. Hence, farmers will face issues in trading the products. Therefore, it is essential to forecast the yield of each crop within a considerable time before harvesting.

It leads to making better postharvest management decisions on the organization of the postharvest value chain within the country. This will help to manage the country's national food demand and food security (Bastiaanssen and Ali, 2003). Predicting the yield before harvesting using spatial distribution is a common practice in novel cropping systems. This Spatial analysis for the paddy lands can be applied to manage food security (Xiao *et al.*, 2005). As rice is the country's staple food, the spatial analysis of paddy is highly convenient for Sri Lanka. Satellite remote sensing can be considered an effective tool for estimating and predicting crop yield (Ferencz *et al.*, 2004). Spatial analysis can provide accurate data about land features. Also, these features can be used to identify the growth of the crops (Liu and Kogan, 2002).

Information acquired from the temporal dynamic remote sensing data closely relates to the identification of plant characteristics. Therefore, it can be applied for yield estimation before harvesting. Satellite images play an important role in providing geographical data (Shahbaz *et al.*, 2012). Quantitative and qualitative information is given by satellite and remote sensing images that reduce the difficulty of fieldwork and research time (Vaiphasa *et al.*, 2011). At periodic intervals, satellite remote sensing technologies capture data/images. The amount of data obtained from data centers is immense (Zheng *et al.*, 2013).Vegetation indices are optical measures of the greenness of the vegetation canopy and directly measure the photosynthetic properties. There is a correlation between the vegetation indices, such as NDVI, calculated based on satellite data and crop yield (Cheng and Wu, 2011).

Using satellite data, the maximum vegetative growth period could be the best time for the paddy yield prediction (Noureldin *et al.*, 2013). The vegetation indices can be used as a yield estimation tool before harvesting (Rasmussen, 1997). Vegetation Indices obtained from canopies based on remote sensing are quite simple and accurate algorithms. It is used for quantitative and qualitative purposes, such as evaluating the vegetation cover and growth dynamics (Xue and Su, 2017). Vegetation indices extracted from this light spectrum range can be attributed to several features outside plants' growth and vigor quantification. The indices are related to water, protein, sugar, pigment, carbohydrate content and aromatic content of crop (Foley *et al.*, 1998);(Batten, 1998).The NDVI is sensitive to the vigor of green vegetation. It is calculated using the visible and NIR light reflected by the target surface. Several studies have found that NDVI performs better in estimating Leaf Area Index (LAI), biomass and other vegetation characteristics when the amount of vegetation increases gradually to a certain value (Major, *et al.* 1990). The DVI is very sensitive to soil context changes. It can be used to detecting the ecological environment of foliage, hence, it is called as "Environmental Vegetation Index (EVI)" (Richardson and Wiegand, 1977). According to the previous studies, the green leaf Chlorophyll absorbed 80–90% of light in the Blue (B) or R portions of the spectrum (Gitelson *et al.,* 2003);(Xiao *et al.*, 2005).

The R and NIR band were found to be the most appropriate portions of the Electro-Magnetic Spectrum (EMS) for assessing crop conditions and biomass. The R spectral interval corresponds to the region of maximum chlorophyll absorption and the NIR spectral interval corresponds to the region of maximum reflectance of incident light by living vegetation (Rouse *et al.*, 1973). Information acquired from the temporal dynamic remote sensing data has a close relationship with the identification of plant characteristics. Therefore, it can be applied to yield estimation before the harvesting. Visible R, G and B bands and NIR regions of the electromagnetic spectrum are successfully used to monitor crop cover and crop yield (Magri *et al.*, 2005);(Baez-Gonzalez *et al.*, 2005). Shanmugam and Dammalage (2018) developed NDVI and EVI2 based models to forecast the paddy yield to the Polonnaruwa district of Sri Lanka.

This study was focused to develop the paddy yield estimation models using satellite data. The objectives of this were to identify the paddy cultivated areas in Kamburupitiya Divisional Secretariat (DS) division using satellite images, calculate the different vegetation indices and reflectance values of G, R and NIR spectral bands of paddy cultivation and validate the developed crop yield models from observed paddy yield data.

Materials and Methods

The research was conducted in Kamburupitiya DS division, Matara district, Sri Lanka during two paddy growing seasons (*Yala* and *Maha*). It is located in Southern province and its geographical coordinates are 6° 5' 0" North, 80° 34' 0" East. Kamburupitiya DS Division (68 km^2 land area) consists 38 Grama Niladhari (GN) Divisions. It provides a significant contribution to the total rice production in Matara District. The Figure 1 shows the map of the study area.

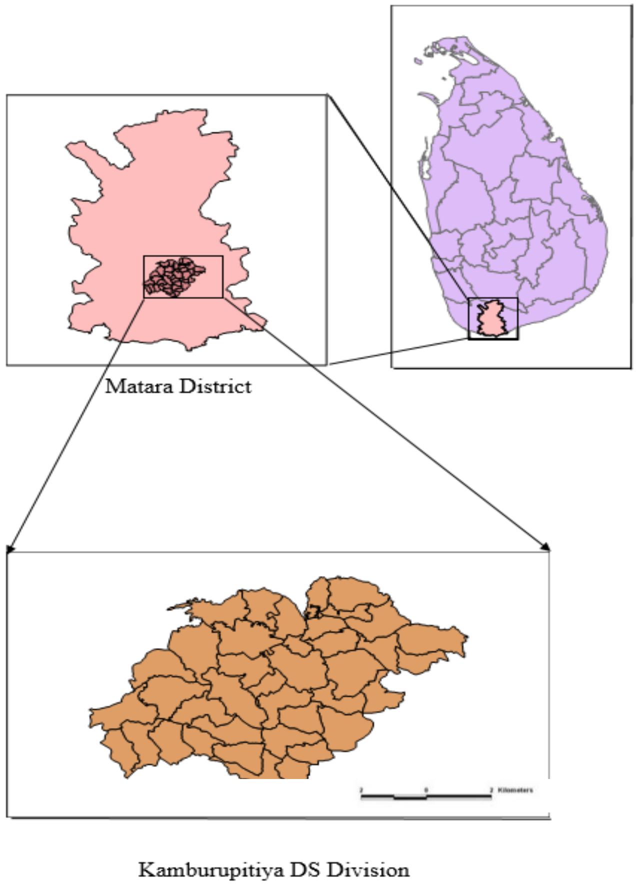


Figure 1: The study area

Three types of data were used as inputs to develop the paddy yield models. They are secondary crop reporting data, satellite data and Google Earth data. Paddy yield data (Crop cutting survey data) and other required local paddy statistics over 5 years in *Yala* and *Maha* seasons from 2016 to 2020 were obtained from Department of Agriculture, Matara. Free satellite images Landsat 8 OLI/TIRS imageries correspond to the years from 2016 to 2020 were selected approximately at the maximum vegetative growth stage of paddy in *Yala* and *Maha* seasons via USGE Earth Explorer Official Website. These Landsat 8 bands have a ground resolution of 30 meters. All images were downloaded from the

USGS Earth Explorer's collection 1 level 1 category and they were belonging to Datum- WGS84 and UTM Zone-44N. The Table 1 shows the acquired satellite images from 2016 to 2020.

Table 1: Acquired satellite images

Date of Acquisition	Path	Row	Scene cloud cover	Land cloud cover
2016.07.05	141	056	17.88	26.30
2017.01.29	141	056	12.27	4.07
2017.06.22	141	056	14.11	38.12
2017.12.31	141	056	22.50	28.99
2018.09.03	141	056	13.08	21.42
2019.01.13	141	056	7.33	6.63
2019.07.30	141	056	13.15	31.07
2020.01.06	141	056	4.62	7.48
2020.08.17	141	056	10.18	26.66

Geo-locations were assessed visually using Google Earth Pro Software application to identify and demarcate the locations of paddy cultivated lands in each growing season in Kamburupitiya DS division. Cultivated paddy lands were identified and marked as polygons (kmz files) in the growing stage of the paddy crops for each season in each year using Google Earth Pro Software Application. Nine images from Landsat 8 OLI/TIRS satellite were used for the time series analysis for both season. Band 1, 2, 3, 4, 5, 6 and 7 of those images were used for the image analysis. Extract by mask were done for each bands using a shape file of Kamburupitiya DS division. The satellite images were atmospherically corrected, as it is necessary for this study. Top of atmospheric corrections and solar zenith angle correction for each extracted bands of acquired Landsat 8 OLI/TIRS images were carried out using the data values of meta data files in each image. Then composite images were created for each season. Paddy polygons (kmz files) which were marked on the Google Earth map were applied as a layer on each composite image as kml files.

The vegetation indices were calculated for the Landsat 8 OLI/TIRS images approximately at the peak of growing stage at each growing season of paddy for all pixels under rice cultivation areas using Esri ArcMap 10.8 version. Five vegetation indices were calculated from different forms of algebraic ratios between R, NIR and G bands. They were NDVI, GVI, RVI, IPVI and DVI using the Raster calculator tool of ArcMap.

NDVI is determined using the R and NIR bands of the given satellite image (Equation 1) (Rouse *et al.*, 1973). R and NIR are spectral reflectance from the R and NIR-bands of the satellite image, respectively. The DVI is expressed in Equation 2 (Richardson and Everitt, 1992).

$$DVI = NIR - RED$$ Equation 1

The GVI was calculated using the relationship between G and NIR bands of the satellite image (Equation 3) (Panda *et al.*, 2010).

$$GVI = \frac{NIR-GREEN}{NIR+GREEN}$$

$$NDVI = \frac{NIR - RED}{NIR + RED}$$ Equation 2

Equation 3

There is a strong correlation with the season's progression between the GVI and grazed pasture green-up (Lecain *et al.*, 2000).

RVI is the ratio vegetation index (Equation 4) (Jordan, 1969), and it can be used to eliminate various albedo effects.

$$RVI = \frac{NIR}{RED}$$ Equation 4

IPVI is the infrared percentage vegetation index (Equation 5)
(Crippen, 1990), and it is determined using the R and NIR bands of the given satellite image.

$$IPVI = \frac{NIR}{NIR+RED}$$ Equation 5

G, R and NIR spectral bands of each satellite images at each
growing season of paddy for all pixels under rice cultivation areas were considered to calculate the reflectance values. Purposive samples of pixel values of the direct spectral data collected from satellite imagery (reflectance values of R, G and NIR bands) and five calculated vegetation indices under the cultivated paddy lands were selected for model building. These pixel values were collected from the paddy cultivated lands in respective GN divisions by crop cutting survey. Then simple linear regression models based on the relationship between the recorded average paddy yield data of Kamburupitiya DS division collected by Department of Agriculture, Matara with the average values of respective Vegetation indices and reflectance values of three spectral bands were developed for both two season using Minitab statistical software. MAPE was used as the statistical indicator for Validation and Accuracy Assessment (Equation 6).

$$M = 1/n \sum_{t=1}^{n} \frac{(At-Ft)}{At}$$ Equation 6

M = mean absolute percentage error

n = number of times the summation iteration happens

At = actual value

Ft = forecast value

For the accuracy validation of the derived models, the average rice yield data of 2020 *Yala* season and 2019/2020 *Maha* season were used, which were not used for modeling. After validating and checking the accuracy of each model, the most accurate models were selected for yield prediction in Kamburupitiya DS division.

Results and Discussion

Development of the paddy yield models

After the cultivated paddy lands were demarcated in the growing stage of the paddy crops for each season in each year using Google Earth Pro Software

Application, the average pixel values of the direct spectral data collected from satellite images (reflectance values of green, red and near infrared bands). Five vegetation indices under the cultivated paddy lands were calculated for the model development process for both *Maha* and *Yala* season.

Linear relationships between paddy yield with the average values of vegetation indices and average reflectance values of G, R and NIR spectral bands have been calculated for respective GN divisions. The regression models on NDVI and RVI for *Maha* Season (Figure 2) showed a higher determinants of coefficient (R^2) values of 77.5% and 76.8%, respectively. The R^2 values with positive relationship were 77.5%, 76.8%, 73.3%, 72.7% 52.1% and 35.9% for NDVI, RVI, GVI, IPVI, DVI and NIR band in *Maha* season, respectively (Table 2. Negative relationship was observed in R and G band with the R^2 values with 73.3% and 46.4 %, respectively.

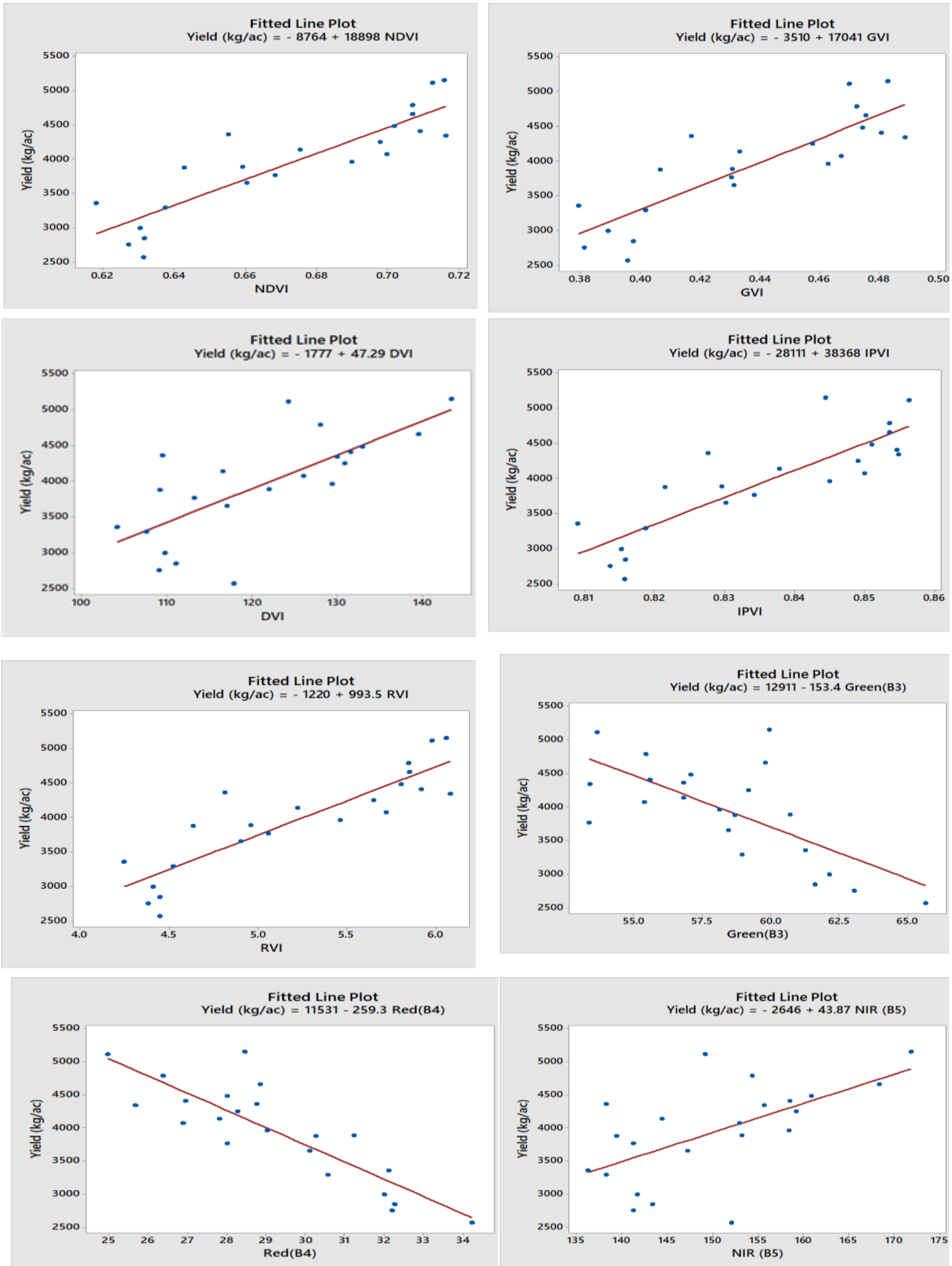
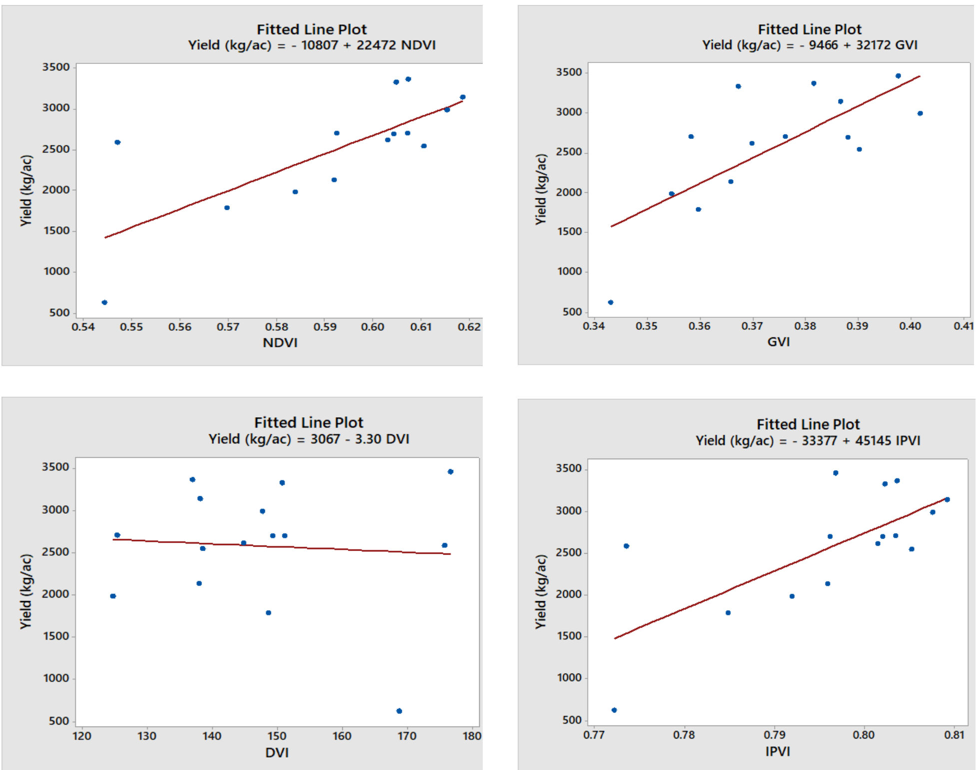


Figure 2: Regression models for *Maha* Season

Linear relationships between rice yield with the average values of vegetation indices and average reflectance values of G, R and NIR spectral bands in *Yala* season are shown in Figure 3. The Coefficients of determination (R^2) of the forecasting models for *Yala* were lower value due to cloud percentages of selected satellite images for *Yala* season were higher than the selected images for *Maha* season. But, the R^2 values of NDVI, RVI, GVI and IPVI based models were higher than 0.5 coefficient of determination as 56.0%, 55.8%, 54.5% and 50.2%, according to the descending order, respectively. Therefore, according to regression equations in this statistical analysis, paddy yield has considerable relationship with NDVI, RVI, GVI and IPVI based models, while DVI, G, R and NIR band models have a lower R^2 value. In this season, the relationships based on NDVI and RVI showed a higher determinant of coefficient (R^2) values than determinant of coefficients of other forecasting models.



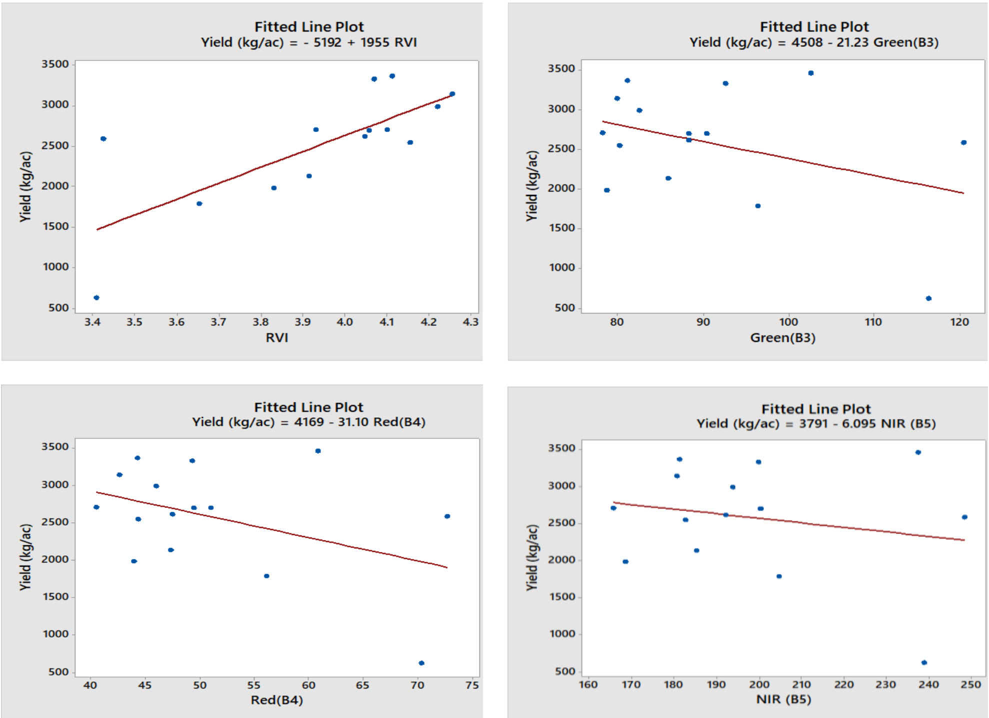


Figure 3: Regression models for *Yala* Season

Validation and accuracy assessment of paddy yield models

Rice yield was predicted for the 2019/2020 paddy cultivated *Maha* season and 2020 *Yala* season based on the derived models to evaluate the yield forecasting. In Tables 2 and 3, the accuracy of validated results is shown as a percentage difference between model-based yield and reported data of Department of Agriculture based on the traditional "Crop-cutting survey" method using MAPE. Other than that, Durbin Watson statistical test was used as an adequacy for validation of these model.

Table 2: Validation of *Maha* Season

Forecaster	Model	R^2 Value (%)	MAPE (%)	Pearson Correlation	P Value	Durbin-Watson Statistic
NDVI	Y= -8764+18898*NDVI	77.5	8.76	0.881	0	1.42093
GVI	Y= -3510+17041*GVI	73.3	9.23	0.856	0	1.81667
DVI	Y= -1777+47.29*DVI	52.1	8.30	0.722	0	1.77318
IPVI	Y= -28111+38368*IPVI	72.7	9.38	0.853	0	1.55955
RVI	Y= -1220+993.5*RVI	76.8	9.88	0.877	0	1.44413
Green	Y= 12911-153.4*Green	46.4	37.36	-0.681	0	1.46014
Red	Y= 11531-259.3*Red	73.3	28.11	-0.856	0	1.60496
NIR	Y= -2646+43.87*NIR	35.9	12.4	0.599	0.003	1.80735

When considering R^2 value, MAPE percentage, Durbin Watson statistics values and Pearson correlation values all together, It was noted in *Maha* season that G, R and NIR band, and DVI as predictors for paddy yield prediction models were not applicable. All other models, with the exception of three spectral bands-based models, have shown less than a 10% difference between model-based (predicted) yield and crop reporting data. Out of those, the GVI-based model had the highest accuracy, with just 9.23 percent of discrepancy and 1.82 of Durbin Watson statistics value. So, according to the results of this research study area, the GVI-based model was the most appropriate one for forecasting rice yield through other yield forecasting models. Hence, the yield model based on GVI values can be selected as the best model for rice yield forecasting using Landsat satellite 8 OLI/TIRS imagery in this particular study area according to this research study for *Maha* season.

Table 3: Validation of *Yala* season

Forecaster	**Model**	**R^2 Value (%)**	**MAPE (%)**	**Pearson Correlation**	**P Value**	**Durbin Watson Statistic**
NDVI	Y=10807+22472*NDVI	56.0	12.72	0.748	0.002	1.87192
GVI	Y=-9466+32172*GVI	54.5	39.27	0.738	0.003	2.20609
DVI	Y=3067-3.3*DVI	0.5	25.28	-0.072	0.799	1.52904
IPVI	Y=-33377+45145*IPVI	50.2	14.68	0.709	0.003	1.61259
RVI	Y=-5192+1955*RVI	55.8	16.72	0.747	0.002	1.933
Green	Y=4508-21.23*Green	14.8	19.15	-0.384	0.157	1.46106
Red	Y=4169-31.10*Red	17.5	15.17	-0.418	0.121	1.45992
NIR	Y=3791-6.095*NIR	4.4	21.60	-0.209	0.455	1.49944

When considering R^2 value, MAPE percentage, Durbin Watson statistics values and Pearson correlation values all together, it was noted in that NDVI, IPVI and RVI as predictors for paddy yield prediction models were applicable for *Yala* season. All these models have shown less than a 20% difference between model-based (predicted) yield and crop reporting data. Out of those, the NDVI-based model had the highest accuracy, with around 12% of discrepancy and 1.87 of Durbin Watson statistics value. So, according to the results of this research study area, the NDVI based model was the most appropriate one for forecasting rice yield for *Yala* Season. Rahman and Robson, (2016) developed yield prediction model derived from maximum Green Normalized Difference Vegetation Index (GNDVI) for predicting sugarcane yield in Bundaberg region using Landsat data with significant correlation with R^2 value of 0.69 and RMSE value of 4.2 t/ha at the peak of the growing stage of the crop.

Conclusion

The yield model based on GVI for the *Maha* season and the NDVI-based model for the *Yala* season can be selected as the best model for rice yield forecasting using Landsat 8 OLI/TIRS imagery at the peak of the growing stage of the paddy. If the limitations are overcome, the models will enable authorities to make successful decisions about rice supply management before the harvesting without incurring high costs and drudgery. The accuracy of the research can be improved using satellite images with high spatial resolution. This study will guide to improve development of prediction models by using Landsat 8 OLI/TIRS satellite imagery time series for paddy yield estimation for the Matara District.

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