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RAPID DETECTION OF AVIAN INFECTIOUS BRONCHITIS VIRUS BY USING COMMERCIAL INSULATED ISOTHERMAL POLYMERASE CHAIN REACTION (iiPCR) AND ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) IN SRI LANKA

Wanasinghe W. M. L. A.[1] and Fouzi M. N. M.[2]*

[1]Department of Animal Science, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Sri Lanka.
[2]Department of Farm Animal Production and Health, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka.

*Corresponding Author: mnmf@vet.pdn.ac.lk (https://orcid.org/0000-0002-6993-7163)
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ABSTRACT

Infectious bronchitis (IB) is considered one of the most important poultry diseases worldwide. Many cases have been presented in Sri Lanka with similar clinical symptoms to those of IB in poultry belt areas. Therefore, the present study aimed to develop the IBV rapid detection methods using commercial insulated isothermal Polymerase Chain Reaction (iiPCR) and enzyme-linked immunosorbent assay (ELISA) in broilers, commercial layers, and backyard poultry flocks in Sri Lanka. Tracheal swabs and blood samples (n=413) were collected from vaccinated (n=107) and unvaccinated (n=306) birds exhibiting clinical signs attributable to IBV. Viral RNA was extracted from tracheal swabs and IBV was detected by iiPCR. Serum was separated from blood samples, and ELISA detected antibodies against IBV. It was reported that 46% (190/413) of birds were serologically positive for IBV by ELISA and 41.88% (173/413) of birds were positive for IBV by iiPCR. The total prevalence of IBV was 20.56% (22/107) of vaccinated and 49.34% (151/306) of unvaccinated poultry flocks by iiPCR. Furthermore, ELISA detected 30.84% (33/107) of vaccinated and 51.30% (157/306) of unvaccinated poultry flocks. Thus, IBV was more prevalent in unvaccinated birds comprised of backyard chickens and broilers in sampled areas. Moreover, the Batticaloa district had the highest prevalence of IBV (>75%). Thus, iiPCR assay could be used for rapid detection of IBV infection in clinical requirements

Keywords: Antibody, ELISA, Infectious bronchitis, iiPCR, Sri Lanka

INTRODUCTION

Infectious bronchitis virus (IBV) is a coronavirus that causes significant economic loss in the poultry industry across the world (Ennaji *et al*., 2020). IBV affects poultry of all ages, resulting in acute respiratory signs such as sneezing, coughing, gasping, nasal discharge, tracheal rales increased morbidity as well as a decrease in egg yield and quality (Cavanagh, 2007; De Wit, 2000). However, routine vaccination against IBV is being done on commercial poultry farms in many countries including Sri Lanka.

The poultry industry is recognized as a rapidly expanding livestock sub sector in Sri Lanka (DAPH, 2022). In many districts of Sri Lanka, poultries especially chickens are reared under the intensive system for egg production (commercial layer), meat production (commercial broiler) and backyard chicken (village chicken) have been reared in Sri Lanka since ancient times (Silva *et al*., 2016; Mokanapriya *et al.*, 2021). Most of the intensive poultry farms are located in Kurunegala and Gampaha areas, while other districts have fewer intensive farms. Districts in the Northern and Eastern provinces of Sri Lanka are known for backyard chicken farming and new intensive farms have also grown up. Jaffna (Northern Province), Trincomalee and Batticaloa (Eastern province) are important districts where poultry farming is now becoming popular with small-scale intensive farming with traditional backyard operations using backyard chickens (JICA, 2012).

Intensive commercial poultry farms are mainly located in two districts of Sri Lanka, namely Kurunegala and Gampaha. However, Jaffna district poultry operations are mainly in small-scale farms, including backyard chicken flocks. Vaccination against IBV is mainly done in intensive farming in Kurunegala and Gampaha. However, despite the continued IBV vaccination on commercial farms, IBV outbreaks are observed in many districts mainly Kurunegala and Gampaha based on the gross lesions as there is no established laboratory close by to confirm IBV by molecular methods. However, IBV has not been extensively studied in Sri Lanka previously. There are limited studies on IB cases in Sri Lanka and Ball *et al*. (2016) reported that over 50% of the IBV positive samples were related to divergent from vaccinal strains in Sri Lanka with his

limited sampling. Furthermore, currently there is no established laboratory facility to confirm IB cases in Sri Lanka. Thus, it is important to know the IBV status in Sri Lanka to take adequate control measures if the disease is present in Sri Lanka.

Virus isolation in embryonated eggs is routinely used to diagnose IB, followed by immunological identification of the isolates (De Wit, 2000) and by molecular identification by PCR (Bijlenga *et al*., 2004). However, the serological tests and the RT-PCR, which are commonly used to detect IBV (Ball *et al*., 2016; De Wit, 2000), are complex and time-consuming laboratory procedures. Moreover, reverse transcription (RT)-PCR, which is a commonly used and prevailing tool to detect IBV (Ball *et al*., 2016; De Wit, 2000), requires specialized procedures such as RNA stabilization and cDNA synthesis, which are time-consuming laboratory procedures. However, PCR techniques have been shown to be very efficient in the detection of IBV (Bijlenga *et al*., 2004). The newly developed insulated isothermal PCR assay (iiPCR) is rapid and sensitive technique to detect many animal diseases (Chua *et al*., 2016; Go *et al*., 2016). This recently developed and widely accessible iiPCR method is based on the fluorescent probe hydrolysis and the detection can be completed in an hour with analytical specificity and sensitivity of up to 10 copies per reaction (Du et al., 2020; GeneReach Biotechnology Corporation (Taichung City, Taiwan). Currently, the iiPCR assays are being developed and used to diagnose several poultry diseases, such as *Mycoplasma* and *Salmonella* (Chua *et al*., 2016; Kubota *et al*., 2013). However, IBV has not been extensively studied using iiPCR in Sri Lanka, previously. Therefore, the present study was aimed at finding the prevalence of IBV in broilers, commercial layers and village/backyard poultry, both in non-vaccinated and vaccinated flocks, using iiPCR technology and ELISA.

METHODOLOGY

Study area and sample collection

Ethical clearance was obtained from the Animal Ethical Review Committee of the Faculty of Veterinary Medicine and Animal Science, University of Peradeniya (Ethical clearance certificate No. VERC-21-03). Suspected cases of IB were selected based on clinical signs such as loss of appetite, coughing, sneezing, nasal discharges, diarrhea, pasted vent, low egg quality and production, postmortem lesions such as haemorrhages in the nephritis, kidney, hemorrhagic tracheitis, caseous or catarrhal exudates in the nasal passages. Tracheal swabs and blood samples were collected from unvaccinated (n=306) and vaccinated (n=107) IB suspected chickens from randomly selected poultry flocks in Jaffna (9.6615° N, 80.0255° E) (Unvaccinated; broilers (n=57) and layers (n=53)), Trincomalee (8.5874° N, 81.2152° E) (Unvaccinated; broilers (n=05) and layers (n=98)), Kurunegala (7.4818° N, 80.3609° E) (Vaccinated; broilers (n=50) and layers (n=57)), and Batticaloa (7.7310° N, 81.6747° E) (Unvaccinated; broilers (n=31), layers (n=12) and backyard chicken (n=50)) districts of Sri Lanka during the period of June 2019 to May 2021. Two milliliters of blood were collected from each bird from the wing venipuncture. Serum was separated from each blood sample and stored at-20^{0}C until further use. Samples were transported to the laboratory on ice and stored at -80^{o}C at the Molecular Laboratory, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya until further processing.

Enzyme-linked immunosorbent assay (ELISA)

The commercial infectious bronchitis virus antibody test kit (AffiniTech Ltd., Bentonville, United States) was used to identify antibodies against IBV in serum samples. The ELIZA antibody test kit (infectious bronchitis virus antibody test kit, Affini Tech ltd) is highly specific for detecting antibodies against all known serotypes of Infectious bronchitis virus in chicken serum. One part sample diluent (4X) was diluted with 3 parts deionized water. Samples were diluted with sample diluent at a 1:400 ratio. A hundred microliters of negative and positive samples were dispensed into duplicate wells. A hundred microliters of diluted test samples were placed in each well of a 96-well micro-ELISA plate and incubated at room temperature for 30 minutes. One part of the wash solution (20X) was diluted with 19 parts of deionized water. After incubation, the ELISA plate was washed three times with 300 µl of wash solution per well, and a hundred microliters of conjugate were dispensed per well. Then, the ELISA plate was incubated at room temperature for 30 minutes. Again, the ELISA plate was washed three more times with wash solution (300 µl) in each well. A hundred microliters of substrate were dispensed into each well and incubated at room temperature for 30 minutes. After incubation, 100 µl of stop solution was added to each well. A microplate reader (BIORAD, Tokyo, Japan) was used to read an ELISA plate at 405 nm and 630 nm wavelengths.

RNA Extraction

Viral RNA was extracted from the tracheal swabs samples by using the Taco^{TM} DNA/RNA Extraction Kit (GeneReach Biotechnology Corporation, Taichung City, Taiwan) according to the manufacturer's protocol. Samples and reagents were loaded into a 96-well extraction plate. The loaded 96-well plate and mixing sleeve/mixing comb were installed by using mini–Automatic Nucleic Acid extraction system (Taco^{TM}, Taiwan) for one hour. The RNA sample was stored at -80^{0}C and used for downstream applications.

Detection of IBV by iiPCR

The POCKIT IBV detection kit manufactured by GeneReach Biotechnology Corporation (Taichung City, Taiwan) uses insulated isothermal polymerase chain reaction (ii PCR) technology to detect the RNA of infectious bronchitis virus (Change et al., 2012; Tsai et al., 2012). This detection kit is specifically designed to be used on an iiPCR compatible instrument, POCKIT™ Nucleic Acid Analyzer (Taiwan). The sensitivity and specificity of iiPCR (POCKIT™ Nucleic Acid Analyzer) are equivalent to real-time PCR, with an analytical sensitivity of up to 10 copies per reaction. The samples were placed into the Nucleic Acid Analyzer for one hour at 520 nm and 550 nm wavelengths.

Statistical analysis

Kappa statistic was used to determine the agreement between the ELISA and the iiPCR in detecting the IBV infection in tested chickens.

RESULTS AND DISCUSSION

Suspected cases of IB exhibited the typical postmortem lesions such as hemorrhages in the nephritis, swollen kidney, salpingitis, undeveloped oviduct with accumulation of clear fluid or purulent, hemorrhagic tracheitis, caseous or catarrhal exudates in the nasal passages (Fig 1). A total of 173 out of 413 sampled birds (41.88%) were positive for IBV by iiPCR and a total of 190 out of 413 sampled birds (46%) were serologically positive for IBV as determined by ELISA test (Fig. 2).

The present study was carried out with sampling in different districts where poultry farming is abundant in Kurunegala, Jaffna, Trincomalee, and Batticaloa districts (Fig. 2). Batticaloa district had the highest IBV prevalence (>75%) in broilers, layers and backyard chickens than other districts. The prevalence of IBV in backyard chickens is high in the Batticaloa district, although backyard chickens possess valuable traits such as disease resistance, adaptation to harsh environments and the ability to utilize poor quality feed (Abeykoon *et al*., 2013). Furthermore, all the broiler chickens and layers were collected from Jaffna, Trincomalee and Batticaloa districts have not been vaccinated, whereas the majority of intensive poultry farms in the Kurunegala district practice strict vaccination programs.

Based on the bird type, IBV screened by iiPCR were highly positive in unvaccinated flocks of commercial broilers, layers and backyard chickens. Thus, the prevalence of IBV in unvaccinated chicken was higher than that of vaccinated chicken in the districts of Trincomalee and Batticaloa except for layers. In contrast to a report in a previous study by Ball *et al*. (2016), the present study shows the prevalence of IBV in broilers was higher in unvaccinated flocks than vaccinated flocks (Fig. 3). This could be due to very closely situated farms with high density in unvaccinated farms than in vaccinated farms and prevalence of virus variants from vaccine strains (De Wit *et al.,* 2011). From a global perspective, it is important to have a better diagnosis to prevent and control the emergence of the avian infectious bronchitis virus and its variants (Dhama *et al*., 2014). In the same context, farmers in the Kurunegala district face serious problems (clinical disease of IB based on the clinical signs and the postmortem lesions) even after the vaccination of different types of vaccines. This could be coupled with a re-detection of vaccinal strains of IBV as per the finding of Ball *et al*. (2016), and De Wit *et al*. (2011). Meanwhile, Ball *et al.* (2016) found an overall 19 out of 34 (55.88%) IBV positive cases as determined by RT-PCR and sequencing in Sri Lanka. The same authors reported higher IBV prevalence in unvaccinated backyard chickens. This could be true as the clinical disease is easily maintained in the unvaccinated population rather than in hyper-immune commercial birds. However, Ball *et al*. (2016) report that extracted RNA from chicken tissues was subjected to RT-PCR to detect the IBV S1 gene, and the product was sequenced. However, iiPCR targets the specific gene and only detects the antigen level of the respective all-vaccine and field virus strains of IBV (up to 10 copies per reaction for iiPCR) in each sample under the wavelengths of 520 nm and 550 nm. POCKIT IBV detection kit uses insulated isothermal polymerase chain reaction (iiPCR) technology to detect the RNA of infectious bronchitis virus (Change *et al*., 2012; Tsai *et al*., 2012). ii PCR is based on qualitative IBV detection. The primers and probes only target specific sequences of IBV.

The ELISA test is a relatively sensitive test to detect early IgG (Chen *et al.*, 2011). The ELISA test was a popular method for detecting antibody responses to IBV infection in chicken flocks (Chen *et al*., 2003; Wing *et al*., 2002). In Taiwan, type-specific antibody detection of local IBV strains was developed to serve as a rapid and reliable diagnostic method for the characterization of IBV clinical infections in the field (Chen *et al.*, 2011).

Our previous study (Fouzi *et al*., 2018) revealed the seroprevalence of avian infectious bronchitis virus in Sri Lanka. In the current investigation, 46% (190/413) of the suspected flocks had high levels of IBV antibody titers measured by ELISA. The present study showed that seropositive of IBV were found by ELISA in unvaccinated flocks of backyard chickens 72% (36/50), layers 46.63% (76/163) and commercial broilers 48.38% (45/93), and in vaccinated flocks of layers 52.63% (30/57) and commercial broilers 6% (03/50). Similarly, Sabrarinath *et al* (2011) also reported that the unvaccinated flocks of backyard

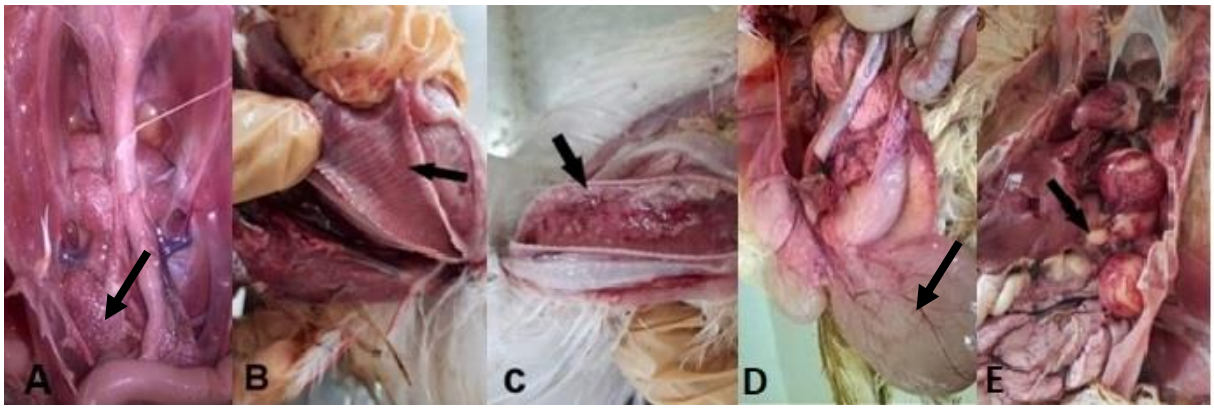

Figure 1: Characteristic lesions in the different organs of chickens suspected of infectious bronchitis based on postmortem lesions such as nephritis and enlarged kidney (A), hemorrhages in the trachea (B), and catarrhal exudates in the larynx (C), salpingitis and fluid filled oviduct (D), and low egg quality and production (E).

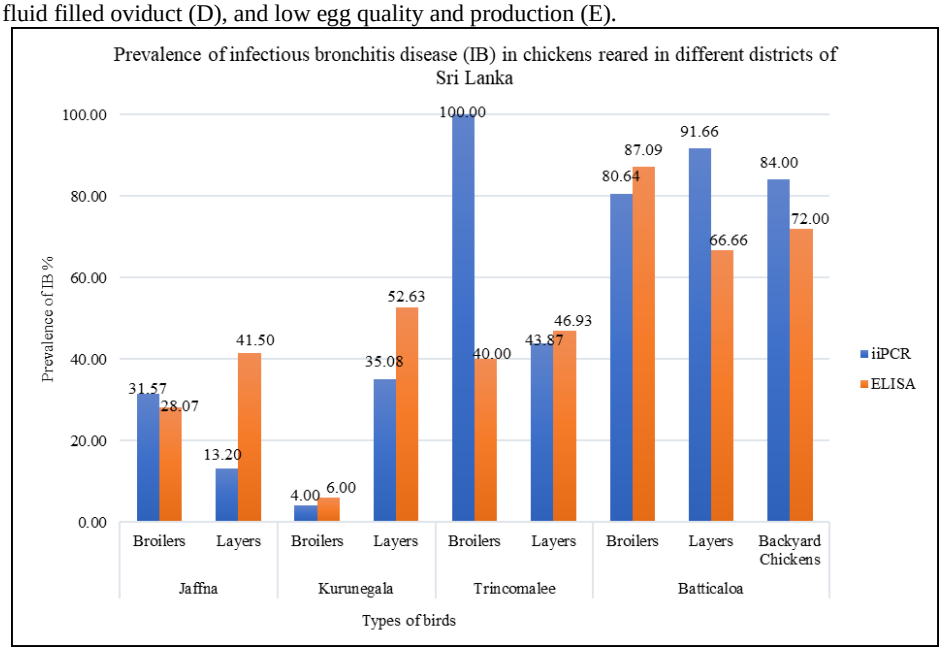

Figure 2: Prevalence of IB in Jaffna, Kurunegala, Trincomalee and Batticaloa districts confirmed by iiPCR and ELISA tests.

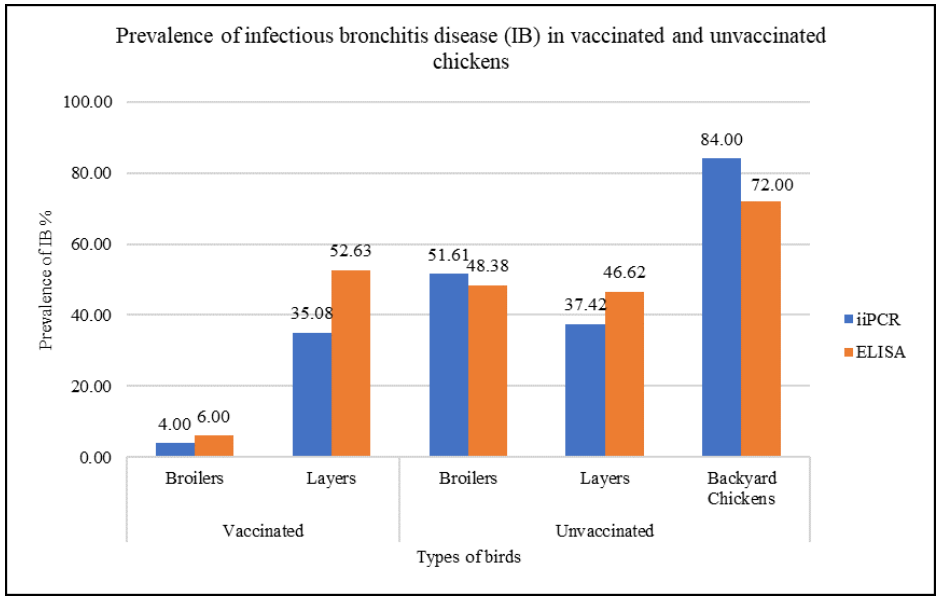

Figure 3: Prevalence of IBV in vaccinated and unvaccinated groups of broilers, layers and backyard chickens confirmed by iiPCR and ELISA tests.

chickens 54.28% (114/210) and 18.02% (31/172) broilers were positive for IBV-antibodies in Grenada. Moreover, unvaccinated flocks of layers 91.67% (176/192) and indigenous chickens 78.32% (177/226), and vaccinated flocks of breeders 90.91% (140/154) were seropositive for IBV in Southwestern Nigeria (Emikpe *et al*., 2010). In Iran, unvaccinated flocks of 82.43% of broilers and 85.3% of backyard hens showed significant anti-IBV antibody titers despite no clinical indications of IBV (Hadipour *et al*., 2011; Mahzounieh *et al*., 2006). Similarly, 88% of commercial poultry flocks were seropositive in Pakistan (Ahmed *et al*., 2007), 92.9% of flocks were seropositive for antibodies in Jordan (Roussan *et al*., 2009), and 82.7% of chickens were seropositive in South Western Nigeria (Emikpe *et al*., 2010). In the present study, a high percentage of seroprevalence was observed in unvaccinated flocks of backyard chickens and vaccinated flocks of layers. This might be an expected finding given the disease's highly contagious nature among Sri Lankan backyard flocks. Moreover, the IBV infection was more prevalent in the backyard chickens and less in the broilers by detection of both iiPCR and ELISA techniques.

CONCLUSIONS

The current investigation found that infectious bronchitis virus (IBV) was found in Sri Lankan poultry flocks, mostly among unvaccinated backyard hens and broilers. It was also observed that vaccinated flocks were also affected with IBV, but lesser extent than unvaccinated flocks. The iiPCR technology could be used for rapid detection of IBV infection in Sri Lanka settings.

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EVALUATION OF GROWTH AND YIELD PERFORMANCE OF CASTOR HYBRIDS UNDER SOUTHERN DRY REGION IN SRI LANKA

Amarasinghe Y.P.J.*, Wijesinghe G., Pushpakumara R.W., Liyanage I.R., and Dilhani R.K.R.

Grain Legumes and Oil Crops Research and Development Center, Angunakolapelessa, Sri Lanka

*Corresponding Author: priyajayantha@gmail.com (https://orcid.org/0000-0002-2044-1140)
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ABSTRACT

Castor oil is considered as a highly important industrial oil due to its unique chemical structure and properties. Although it is not yet commercially grown in Sri Lanka, there is a high potential of promoting castor cultivation in marginal lands. Due to growing interest among the private sector to initiate commercial scale cultivation, there is an immediate necessity to introduce promising castor varieties despite traditional varieties are presently grown. With this objective two castor hybrids, namely; Jiaxiang No.6 (JX-6) and Zibi No.5 (ZB5) were received from China and field evaluation was carried out at Grain Legumes and Oil Crops Research and Development Center, Angunakolapelessa (GLORDC) during 2021/22 Maha season. The experiment was layout in a Randomized Complete Block Design (RCBD) with four replicates, where a local cultivar was used as a check variety. The results revealed, plant height at flowering was not significantly different (P=0.05) among the tested genotypes. Since the local cultivar is having a perennial growth habit, significantly higher (P=0.05) branching was observed. The number of racemes per plant was significantly high (P=0.05) in the local cultivar, while average raceme length and number of fruits per raceme were significantly high (P=0.05) in both hybrids. Additionally, hybrids had higher 100 seed weight ranging from 20.82-23.1g. Hence, a significantly higher (P-0.05) seed yields has been revealed by the hybrid varieties Interestingly, both hybrids showed severe disease susceptibility specially for fusarium wilt, in which the local cultivar showed high degree of resistance. Considering the yield performance, only the hybrid of Jx-6 was selected as a promising hybrid to initiate commercial scale cultivations.

Keywords: Castor, Sri Lanka, Evaluation, Growth, Yield

INTRODUCTION

Castor (*Ricinus communis* L.) is a diploid (2n = 20) oil crop which belongs to family *Euphorbiaceae* that is found across tropical and semi tropical regions of the world. Globally, castor oil is considered as highly important in the chemical industry, since it is the only commercial source of a hydroxylated fatty acid (Severino et al, 2012). Castor oil is a non-drying oil with high viscosity, in which is not easily clogged even under a temperature as low as -56 C (Patel et al, 2016). Moreover, it is a pale-yellow liquid, which contains high amount of recinoleic acid. It is widely used as a starting material for many industrial chemical products because of its unique structure. Hence, it is used in many industries such as cosmetics, surface coatings, toiletries, pharmaceuticals, perfumes, soaps, lubricants, paints, medicines, etc.

Castor is widely grown on arid and semi- arid areas of the world, where India is the main castor producing country in the world. In Sri Lanka, there are several naturally grown perennial castor types. Traditionally, castor oil is used for medicinal purposes specially in ayurvedic medicine in Sri Lanka. Under the local climatic conditions, castor can be grown throughout the year. Since castor can tolerate in unfavorable environmental conditions such as drought, salinity and low soil fertility, there is a potential of promoting castor in such marginal lands in Sri Lanka.

The global castor oil market has reached a volume of 740.5 kt which is expected to be expanding. Currently, China is the main importer of Castor. During the last decade, the interest in castor cultivation has escalated due to the global demand of castor oil, where many countries are making serious efforts to commercialize the castor cultivation (Anjani K, 2014).

Castor reproduces predominately (80%) by cross pollination, in which the primary pollinating agent is the wind (Milani and Nobrega, 2013). Therefore, great variation in phenotypic expression is observed in wild populations of castor. Cultivar/variety development of castor is practiced through several breeding techniques such as mass selection, conventional sexual hybridization and selection, recurrent selection and hybrid development. The advantages of hybrids over cultivars have resulted growing areas predominately with hybrids.

Currently in Sri Lanka, there are no elite cultivars available since there were no efforts to cultivate castor in a commercial scale. Recently there is some interest among private sector to initiate castor cultivation as an industry. Hence, there is an immediate necessity of introducing exotic varieties/hybrids to the country. With this objective we have received two exotic hybrids from Republic of China for the testing of adaptability under local condition. The plant growth and yield performances of the hybrids were compared with a locally available cultivar in order to recommend hybrids for commercial cultivation in Sri Lanka.

METHODOLOGY

Experimental site

The Field trial was conducted at an isolated research field of Grain Legumes and Oil Crops Research and Development Center, Angunakolapelessa, Sri Lanka (GLORDC) which belongs to DL_{2b} agro-ecological zone during 2021/22 *Maha* (from October 2021 to March 2022) season.

Experimental materials

Seeds of two castor F1 hybrids namely Jiaxiang No:6 (JX-6) and Zibi No:5 (ZB-5) which developed in China were received from Pearlead International (Pvt) Ltd, Sri Lanka. A local cultivar was used as a control to compare the performance of two hybrids. These two hybrids were initially selected by considering the climatic factors in Sri Lanka. The local cultivar was the predominant cultivar available in the country which is phenotypically having reddish color stems.

Experimental design

Two imported hybrids (JX-6 and ZB-5) along with the local cultivar as the control were layout in a field experiment in a Randomized Complete Block design (RCBD) with four replicates with a plot size of 6 x 4.5 m. Each plot was prepared as raised beds in which 3-4 seeds were planted in each hole with a spacing of 90 cm between rows and 60 cm within row. Thinning out was carried out in two weeks after emergence keeping only one vigorous plant per hill.

General agronomic practices

Since there were no recommended agronomic package for castor in Sri Lanka, following agronomic practices were carried out considering the recommendation for the hybrid castor cultivation in China. Mainly, Basal fertilizer was added to the soil with a rate of Urea- 135 Kg/ha, Triple Super Phosphate (TSP)- 138.75 Kg/ha and Muriate of Potash (MOP)- 101.25 Kg/ha. Additional urea was applied in a rate of 150 Kg/ha, during the flowering stage. Additionally, manual weeding was done once each at growth stages such as seedling, flowering and harvesting. Supplementary irrigation was provided whenever required.

Collection of data

Total of five plants in each plot avoiding the border plants were tagged for data collection. The main growth and yield parameters were recorded (Table 01) partly considering the characterization of castor described in Da silva et al 2019. Moreover, the number of plants showed a fungal disease symptom of wilting (The exact disease was not formally identified) at first two harvesting periods were recorded.

Table 01: Description of evaluation methodology of plant growth and yield parameters

Descriptor	Evaluation
Plant height at flowering	Measurement from the soil to the first point of the primary raceme
Stem diameter at flowering	The middle third of the stem, using a digital caliper
Number of branches/plant	Count the number of branches at 60 days after emergence
Number of racemes/plant	Count the number of racemes in each plant at 90 days after emergence
Average raceme length	Measure using a ruler in first three racemes of the plant and calculate the average.
Number of fruits/raceme	Count the number of fruits in first three racemes and calculate the average
Mean seed yield	Seed yield of plants in the middle plants of each plot up to 120 days after emergence was measured and converted to per hectare yield.
100 seed weight	Determine the weight of 100 random seeds at 9% moisture level.
Oil yield (%)	Extracted using Soxhlet method in a 1g of dry seed sample.

Data analysis

Rain fall data during the cropping period was obtained from the climatology unit of GLORDC in order to compare the initial growth of the tested genotypes. The growth and yield data were subjected to ANOVA using SAS statistical package version 9.1.3 and the least significance difference (LSD) was used to compare the means.

RESULTS AND DISCUSSION

Both hybrid varieties showed a rapid initial growth rate (seedling) in comparison to the local cultivar. Interestingly, both hybrids were not uniform, where several plant characters such as stem color, presence of stem wax were showed a variation in the population.

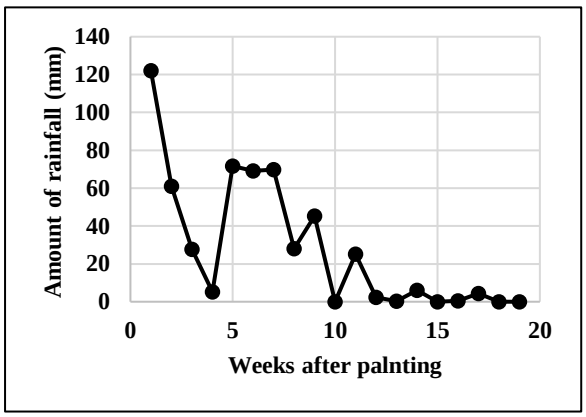

Figure 01: Weekly rainfall (mm) from date of planting (2021/10/22) to end of February 2022

Heavy rainfalls were observed during the initial stage of the crop, which may have affected the optimum crop growth. Due to these frequent rainfalls, water table in the experimental plots were above normal during that period (Figure 01).

Table 02: Variation in major plant growth parameters among the tested castor lines

Hybrid / Cultivar	Ph at fl. (cm)	Sd at fl. (mm)	NoB /plant (at 60 DAE)	NoD to first pick from seedling emergence
ZB-5	50.67a	19.75ab	2.72b	75
JX-6	56.30a	21.62a	3.16b	75
Local cultivar	46.12a	15.50b	9.30a	75
LSD	17.25	5.79	2.54	
CV %	19.5	17.6	29.1	

Within each column, the means followed by the same letters are not significantly different p = 0.05; Ph-Plant height; DAE-Days After Emergence; Sd–Stem diameter; NoB-No. of Branches; NoD-No. of days; fl-flowering

Plant height at the flowering stage were ranged between 46.12 cm to 56.30 cm (Table 02) among the three genotypes and were not significantly different (P=0.05). Stem diameter at flowering showed a significant variation, where two hybrids had higher stem diameter at flowering stage than the local cultivar. Interestingly, higher branching habit was observed in the local cultivar in comparison to the hybrids indicating determinate growth habit in the hybrids (Table 02).

According to the visual observations disease severity was increased with the water stress.

Variability of major yield parameters in the tested castor lines are listed in the table 03. Number of racemes per plant were significantly higher (P=0.05) in the local cultivar while the average raceme length and number of fruits per raceme was significantly higher in the two hybrids. Considering the mean seed yield, both hybrids have performed better than the local cultivar. The mean seed yield of two hybrids were in the range of 1487.3-1987.9 Kg/ha in 4 to 5 picks. Since the number of racemes per plant is high in the local cultivar, the frequency of harvesting is high. Further, local cultivar can be harvested for a long period of time. The ratooning ability of hybrids need to be studied in order to understand the performance for a long period of time.

Table 03: Variation in the major yield parameters

Hybrid / Cultivar	No: of raceme/ plant	Average raceme length (cm)	No: of fruits /raceme	Mean seed yield (Kg/ha)
ZB-5	2.7b	46.80b	61.85b	1487.3a
JX-6	3.9b	58.85a	78.47a	1987.9a
Local cultivar	9.4a	35.92c	31.02c	878.3b
LSD	1.50	9.58	7.98	571.24
CV %	16.29	11.73	8.08	17.36

Within each column, the means followed by the same letters are not significantly different p = 0.05.

According to the visual observations of the genotypes to the abiotic stresses, local cultivar has more ability than two hybrids to tolerate water stress.

Both hybrids showed larger seed size, in which the 100 seed weight were ranging 20.82 to 23.11 grams. In comparison, local cultivar had low 100 seed weight (Table 04). Considering the oil yield in the tested lines, the local cultivar showed the highest while the oil content of exotic lines had comparatively lower value (Table 03).

Table 04: 100 seed weight and oil yield of tested lines

Hybrid / cultivar	100 seed weight (g) ± SD	Oil yield (%) ± SD
ZB-5	20.82 ± 1.07	44.48 ± 3.35
JX-6	23.11 ± 0.76	53.43 ± 4.74
Local cultivar	8.19 ± 0.46	60.17 ± 4.68

SD- Standard deviation of mean (n=4).

Considering the response to biotic stresses, no severe pest damages were observed. During the vegetative stage several plants were attacked by leaf eating caterpillars and leaf minors, which were controlled by chemical applications.

Interestingly, during the harvesting stage hybrid plants showed wilting of plants, probably caused by soil borne fungal organism to the root system.

Table 05: Number of plants observed with disease symptoms at two harvesting stages

Hybrid/ cultivar	Mean value of wilted plants at first harvest (Total plants/plot-50, n=4)	Mean value of wilted plants at second harvest (Total plants/plot-50, n=4)
ZB-5	13.5	18.7
JX-6	7	11.2
Local cultivar	0	0

Both exotic hybrids were susceptible to the disease, although disease severity was high in variety ZB-5, in which JX-6 has shown some field tolerance. Interestingly, local cultivar was highly resistant to the disease (Table 05). Further studies need to be carried out in order to understand the causal organism of the disease.

CONCLUSIONS

Global Castor oil industry is on an expanding trend, in which a secure market would be the highest concern in planning a commercial cultivation in Sri Lanka. Although, there are abundant wild cultivars in the country, currently no distinct variety or cultivar has been identified. Therefore, it is important to identify promising cultivars/varieties in order to initial castor cultivation at commercial level.

The two exotic hybrids had superior performance in terms of the yield parameters such as average raceme length, number of fruits per raceme and seed weight. Therefore, both hybrids have given significantly high seed yield over the local cultivar. Interestingly, considering the disease incidences, local cultivar had shown superior characteristics. Since, commercial scale cultivation can be initiated with exotic hybrids as they have high yield potential, it is important to incorporate the beneficial characteristics of local cultivars in order to develop well adapted castor varieties in the future.

Both commercial hybrids have performed better than the local cultivar tested, in terms of the seed yield. Overall, exotic hybrid; Jiaxiang No:6 (JX-6) has performed well under field conditions. Further, disease severity was comparatively low in hybrid; Jx-6 and proper cultural practices can be very important in a commercial scale cultivation.

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EFFECTS OF DIFFERENT ORGANIC APPLICATIONS ON GROWTH AND YIELD OF *Raphanus sativus* (RADDISH)

Vidanapathirana N.P.*

Department of Agro-Technology, University of Colombo Institute for Agro-Technology and Rural Sciences, Hambantota, Sri Lanka

*Corresponding Author: nisansala@uciars.cmb.ac.lk (https://orcid.org/0000-0003-1187-5681)
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ABSTRACT

Radish is an edible root vegetable belonging to the Brassicaceae family. It is a vegetable that matures quickly and is simple to grow. More farmers are switching to use of organic manure as a result of rising knowledge of the risks associated with chemical fertilizers. A field experiment was conducted at the University of Colombo Institute for Agro-Technology and Rural Sciences in Hambantota, Sri Lanka, to evaluate the effects of several organic preparations on the growth and yield of radish. Thirteen treatments were replicated four times following RCBD design. Different organic preparations namely Newly Modified Panchagavya (NMPG), fish tonic, compost tea and a commercially available preparation of Crop Master were used in various concentrations based on the previous research findings and recommendations. Water was applied as a control. Data on plant height, number of leaves, leaf length (cm), leaf width (cm), fresh leaf weight (g), fresh root weight of plant and total yield per treatment (Kg/ha) were recorded and analyzed statistically using ANOVA in SAS 9.1.3 package. The treatment means were compared using DMRT at 5% significance level. The results revealed that, application of NMPG had a significant higher effect on most of the tested parameters. Further, 4% of the NMPG showed better values compared to other concentrations. It was followed by the 3% and 2% of NMPG. Compost tea and crop master followed values next to NMPG. Based on the present findings it can be concluded that, Newly Modified Panchagavya can be used as organic application to cultivate Radish plants with a better growth and yield to obtain potential yield of radish.

Keywords: Newly Modified Panchagavya, Compost tea, Fish tonic, Organic preparations, Radish

INTRODUCTION

The edible section of radish contains 14.8 mg of vitamin C and a range of other nutrients. Othiocyanate is what gives radish its distinctively sour flavor (Kushwah, 2016). It is used to treat piles, piles-related insomnia, persistent diarrhea, and neurological migraines (Singh and Bhandari, 2015). Radish growth and yield are highly reliant on soil and climate factors. For each variety to perform at its best, the soil and climate conditions must be varied. One of the key agro techniques that affects the growth and production of radish is nutrition. The crop's nutritional needs are influenced by soil type, soil fertility, agroclimatic conditions, and variety. The root growth should be quick and unbroken because this crop has a short lifespan and grows quickly. Therefore, optimal fertilization using organic, inorganic, and biofertilizers is necessary to yield good grade radish (Dhanajaya, 2007).

To increase the production of vegetables, excessive amounts of chemical fertilizers are used. Negative consequences on soil resulted from a complete reliance on inorganic fertilizers. Due to growing public awareness, there is an increase in the demand for organic food (Bhatta et al., 2008). In 2018 saw a global market for organic food reach $100 billion USD. But there is a gap between the supply and demand of organic food. In 2017, the percentage of organic vegetable production in overall vegetable production was 1.1%. (Willer and Lernoud, 2019).

It has become necessary to use integrated nutrient management, which uses organic sources like farmyard manure, vermicompost, poultry manure, and neem cake, in order to address the issue of the high cost of chemical fertilizers, meet crop nutrient requirements from a single source, and address the issue of soil health. The application of these organic manures to increase crop production and preserve soil fertility and productivity has gained popularity in recent years. Positive effects of organic manure on soil's water-holding capacity and texture (Kale et al., 1991).

There are many different organic applications on the market, however, there are not quality products to be used. Further, it lacks the knowledge and research findings to use them effectively. In order to determine how various organic applications affect for radish growth and yield, this study was carried out. Its specific objective was to ascertain how does the effective percentage of organic application effect on growth and yield parameters of Radish.

METHODOLOGY

A field study was conducted at the farm field of University of Colombo Institute for Agro-Technology and Rural Sciences, Weligatta, Hambantota, Sri Lanka from 2022 March to May 2022. The field was ploughed and the ground was levelled before preparing the beds. The length and width of a bed was 1m x 1m. Seeds were planted in beds with the spacing of 10cm x 30cm between plants and rows. Watering was done daily.

Application of organic applications

One and a half weeks after planting the seeds, organic liquid applications were applied to the beds. After that, they were applied once a week. When applying treatments, it was applied 500 ml per bed. Application has done as a foliar application.

Treatment structure

The field Experiment was laid out in Randomized Complete Block Design (RCDB) with 13 treatments and four replicates in each. Various organic preparations with different concentrations were used as treatments as follows;

T1 – Newly Modified Panchagavya (NMPG) 2%

T2 - NMPG 3%

T3 - NMPG 4%

T4 – Fish tonic 5%

T5 – Fish tonic 10%

T6– Fish tonic 15%

T7 – Compost tea 30%

T8 – Compost tea 40%

T9 – Compost tea 50%

T10 – Crop master 2%

T11 – Crop master 3%

T12 – Crop master 4%

T13 – Control (Water)

Preparation of the fish tonic (for 5L)

Fish tonic was prepared using 6kg of fish waste and 2kg of sugar to prepare 5 Litres. Fish waste was chopped and added into a plastic bucket and 2kg of sugar was added to the mixture. Then it was mixed once a week and maintained for 2 weeks and then 1 Litre of water was added. Then the mixture was allowed for fermentation for one month. Fermented fish tonic was applied as fertilizer by diluting with water.

Preparation of compost tea (for 150L)

The ingredients used to prepare the compost tea were; 5kg of compost and 250g of sugar. The ingredients were mixed with chlorine free water amount of 150 Litre and it was kept for 7 days while aerating. Later, it was filtered into another container and stored at room temperature in darkness.

NMPG and Crop Master

Commercially available NMPG and Crop Master were used for the experiment.

Analysis of organic preparations

Fish tonic, NMPG , Crop Master and compost tea were tested for its proximate components such as N, P, K, pH and EC.

Data collection

Plant height, number of leaves, length and width of leaves were taken as growth parameters from 40 plants leaving border rows. After harvest, the weight of the plant, the weight of the tuber and total yield were recorded as yield parameters in 40 plants / treatment.

Plant height (cm) was measured in cm using a measuring scale from the soil surface to the end of the leaf. Number of leave per plant were observed in ten plants of each plot. Each leaf was measured after reaching the maximum length, from the sharp section at one end to the point where it joined the stalk at the other, using a ruler. A line of intersection between the cross section and the leaf width serves as the point at which the leaf width is measured to obtain average leaves size (length, width). After harvesting, the weight of the whole radish plant was measured to obtain fresh weight of the total plant and the weight of the leafy part of the radish plant was measured to get Fresh weight of the above ground part. The weight of the root part of the radish plant was measured to obtain fresh weight of the below ground part

Soil Analysis

Random soil samples were taken from the field before the experiment. The sample was kept in a polythene bag. The sample was afterwards dried at room temperature before being sent to TRI Galle for examination of the soil's chemical properties, including its pH, EC, N, P, and K levels.

Analysis of the NMPG, Fish tonic, Crop master and Compost Tea

The nutrient values N, P, K, pH and EC of NMPG manure and fish tonic, Crop Master and compost tea manure were examined.

Data Analysis

To determine the significance at the treatment level, the measured data were statistically evaluated using the Analysis of Variance (ANOVA) using the SAS program version 9.1. The Duncan Multiple Range Test (DMRT) was used to compare the variance between treatment means.

RESULTS AND DISCUSSION

Plant height

It was found that, there were significant differences between the treatments on different organic applications on plant height at 4th week. The higher plant heights were obtained at NMPG in 2% and 3% concentration levels. NMPG was followed by the compost tea and crop master in measured variable. According to Kumaravelu et al. (2009), growth was encouraged at two under Panchagavya irrigation. In almost all of the treatments, Panchagavya encouraged epicotyl extension. The plant height and seedlings' fresh and dry mass are often reserved for panchagavya irrigation. The comparable observations have been made by Muthuvel (2002). In Green gram. The plants significantly doubled in size in a pot study after receiving a 3% Panchagavya spray at ten days after application. At 3% and 4% treatment, the number of nodules, fresh and dry mass, and lateral roots of the plants greatly increased. Growth matched that of management at 5% foliar spray. Additionally, 2% and 3% Panchagavya spray were added, doubling the plant's total leaf area. The number of branches per plant and plant height increased after four Panchagavya sprays at 3%, according to The results of the current research showed that foliar spraying of NMPG at 3% significantly improved yield attributes. The findings of Birendra and Christopher 2007 and Swaminathan et al., 2007 are also in agreement with this observations for Black Gram.

Number of leaves

It was found that, there were significant differences between the treatments on different organic fertilizers on number of leaves. The higher number of leaves was observed in Newly Modified Panchagavya applied with various concentrations. The number of leaves did not significantly alter as the leaf concentration was increased. According to a study by Velmurugan (2005), seed treatment with foliar spray of panchagavya (3%), as opposed to other treatments, resulted in plants that were taller (26.75 cm) and had more leaves (14.0). This was further underlined by Somasundaram and Singaram (2006), who discovered that panchagavya spray had a favorable impact on the development of numerous field crops.

Leaf length

It was found that, there were significant differences between the treatments on different organic fertilizers on Leaf length 4th week. The highest was obtained at Newly Modified Panchagavya 4% concentration level. Increasing the concentration of Newly Modified Panchagavya showed a significant effect on length of the leaves.

Leaf width

It was found that, there were significant differences between the treatments on different organic fertilizers on Leaf width 4th week. The higher values in leaf width were obtained at Newly Modified Panchagavya with 3% and 4% concentration levels. Somasundaram and Amanullah have reviewed and documented the good effects of panchagavya on crop growth and productivity (2007). According to Papen et al. (2002), panchagavya contains phosphobacteria, azotobacter, and azospirillum. In addition to minerals, Panchagavya also contains microorganisms that promote plant growth. According to Sutar et al. (2019), panchagavya spray at 7.5% resulted in noticeably greater growth of the cowpea.

It was found that, there were significant differences between the treatments on different organic applications on plant bio mass. The highest value was obtained at Newly Modified Panchagavya at 4% concentration level.

According to Tharmaraj et al. (2011), the use of Panchagavya significantly increased the number of pods/plant, the number of seeds per pod, the yield of grains, and the look-at weight by 20, 7, 4.2 Kg, and 3.9 g in comparison to NPK and management. The outcomes for the NPK-treated plants were marginally worse to those for the Panchagavya-treated plants. The large improvement in nitrogen content, chlorophyll content, and dry matter accumulation that was demarcated higher than may be attributed to Panchagavya's greater yield and yield qualities In addition, Selvaraj (2003) discovered a 360% increase in French bean yield when Vermicompost and Panchagavya were used. Due to an increase in the biological potency of crop plants, Natarajan (2002) reported that Panchagavya spraying increased the yield of agricultural plants.

Leaf weight

It was found that, there were significant differences between the treatments on different organic fertilizers on leaf weight. The highest was obtained at the Newly Modified Panchagavya 4% concentration level. It was followed by the other tested concentration levels of Newly Modified Panchagavya. Plants sprayed with panchagavya invariably produce larger leaves and create a denser canopy, according to Somasundaram and Amanullah (2007); Tharmaraj et al. (2011).

Table 1: Proximate analysis of organic preparations

Parameters	NMPG	Fish tonic	Compost tea	Crop Master
pH	3.37	4.40	6.50	6.70
EC	-	9.48	1.50	1.40
N	2.76	Not detected	0.03%	2.2%
P	0.25%	2.80%	Not detected	1.5%
K	2.41%	1.28%	0.17%	2.00%

Above table 1 is indicating the pH, EC, N, P and K components of the used organic preparations.

Table 2: Effect of different organic applications on growth parameters. Plant height, number of leaves, leaf length and leaf width (Mean values of 40 plants)

Treatment	Plant height cm	Number of leaves	Leaf length cm	Leaf width cm
T1	15.33 ± 0.02^{a}	10.17 ± 0.05^{a}	11.06 ± 0.02^{c}	5.91 ± 0.01^{b}
T2	15.53 ± 0.72^{a}	10.20 ± 0.16^{a}	11.20 ± 0.13^{b}	5.72 ± 0.48^{a}
T3	13.05 ± 0.02^{b}	10.32 ± 0.12^{a}	11.33 ± 0.02^{a}	5.98 ± 0.00^{a}
T4	7.94 ± 0.01^{cd}	5.50 ± 0.08^{e}	6.63 ± 0.03^{g}	3.61 ± 0.00^{d}
T5	7.96 ± 0.01^{cd}	5.50 ± 0.11^{e}	6.64 ± 0.01^{g}	3.62 ± 0.01^{d}
T6	7.98 ± 0.00^{cd}	5.52 ± 0.09^{de}	6.66 ± 0.01^{g}	3.63 ± 0.00^{d}
T7	9.81 ± 0.02^{c}	5.72 ± 0.12^{c}	9.58 ± 0.05^{e}	4.30 ± 0.00^{c}
T8	9.88 ± 0.01^{c}	5.80 ± 0.08^{c}	9.60 ± 0.00^{de}	$4,32\pm0.00^{c}$
T9	9.95 ± 0.01^{c}	5.85 ± 0.05^{bc}	9.66 ± 0.01^{d}	4.33 ± 0.02^{c}
T10	9.44 ± 0.06^{c}	5.52 ± 0.22^{de}	8.67 ± 0.05^{f}	4.20 ± 0.01^{c}
T11	9.47 ± 0.06^{c}	5.70 ± 0.00^{cd}	8.65 ± 0.07^{f}	4.23 ± 0.00^{c}
T12	9.48 ± 0.06^{c}	6.00 ± 0.08^{b}	8.68 ± 0.09^{f}	4.22 ± 0.02^{c}
T13	6.93 ± 0.02^{d}	5.22 ± 0.05^{f}	4.94 ± 0.01^{h}	3.52 ± 0.01^{d}
Sig.	*	*	*	*

The values indicate the mean ± standard error of four independent samples. According to DMRT, means with identical superscripts in the same column are not statistically different at the 0.05 probability level. "" and "ns" indicate significance at P0.05 and non-significance, respectively.*

Table 3: Effect of different organic applications on yield parameters

Treatment	Leaf Fresh weight/plant (g)	Root fresh weight/plant (g)	Yield (Kg/ha)
T1	131.48 ± 0.11^{c}	95.50 ± 0.00^{b}	22600.98 ± 0.11^{c}
T2	136.80 ± 0.53^{b}	96.10 ± 0.06^{a}	23200.90 ± 0.60^{b}
T3	138.56 ± 0.28^{a}	96.56 ± 0.02^{a}	23500.16 ± 0.29^{a}
T4	45.00 ± 0.17^{g}	85.46 ± 0.40^{e}	13000.48 ± 0.57^{f}
T5	45.30 ± 0.15^{g}	86.08 ± 0.08^{d}	13100.39 ± 0.22^{e}
T6	45.21 ± 0.04^{g}	87.45 ± 0.28^{c}	13200.70 ± 0.26^{d}
T7	63.15 ± 0.03^{d}	40.23 ± 0.46^{h}	10300.38 ± 0.45^{j}
T8	63.20 ± 0.00^{d}	40.62 ± 0.07^{h}	10300.82 ± 0.07^{j}
T9	63.22 ± 0.02^{d}	40.71 ± 0.69^{h}	10300.89 ± 0.37^{j}
T10	51.71 ± 0.03^{f}	53.68 ± 0.03^{g}	10500.42 ± 0.38^{i}
T11	51.90 ± 0.06^{f}	54.61 ± 0.23^{f}	10600.51 ± 0.28^{h}
T12	52.51 ± 0.48^{e}	55.15 ± 0.02^{f}	10700.66 ± 0.49^{g}
T13	30.07 ± 0.59^{h}	23.20 ± 0.25^{i}	5300.28 ± 0.18^{l}
Sig.	*	*	*

The values indicate the mean ± standard error of four independent samples. According to DMRT, means with identical superscripts in the same column are not statistically different at the 0.05 probability level. "" and "ns" indicate significance at P0.05 and non-significance, respectively.*

Root weight

It was found that, there were significant differences between the treatments on different organic fertilizers on root weight. The higher values in root weights were obtained at 3% and 4% concentrations of Newly Modified Panchagavya. This was proved with several previous studies also. *Abelmoschus esculentus* yield parameters were multiplied in three times panchagavya spray when compared to control and varied concentrations, claim Rajasekaran and Balakrishanan (2002). According to Tharmaraj et al. (2011), the use of panchagavya improved production and disease resistance in a variety of pulse plant species, including Radish, *Vigna mungo*, *Arachis hypogea*, *Cyanopsis tetragonoloba*, *Lablab purpureus*, *Cicer arietinum*, and *Oryza sativa*. According to Boraiah et al. (2017), the use of Jeevamrutha, cow urine, and Panchagavya half-percent spray resulted in significantly higher fruit yields than other organic liquid formulations.

According to Kanimozhi et al. (2003), Panchagavya application at 4% spray was discovered to be superior in terms of root yield. Furthermore, Suchitra Rakesh et al. (2017) noted that when *Abelmoschus esculentus* was sprayed with different concentrations of panchagavya, yield parameters including fruit weight and number were presented. When compared to control and other concentrations, the plants sprayed with 3% concentration of panchagavya produced the highest yield parameters, including fruit weight and number (30.67 mg/fruit).

Subramanian et al. (2005) claim that employing panchagavya increases yield, improves product quality, is less expensive, and is more environmentally friendly with no negative side effects. When compared to control and other concentrations, the yield metrics of Abelmoschus esculentus (fruit number and weight) were increased with 3% panchagavya spray. Similar results were seen in *Vigna mungo*, *Oryza sativa*, black and green grams (Brito and Girija, 2006), groundnut, and *Vigna mungo* and *Oryza sativa* (Rajasekaran and Balakrishanan, 2002). (Ravikumar et al., 2012). Panchagavya improves the keeping quality of vegetables and fruits as well as the growth and vigor of crops by fostering resistance to pests and illnesses (Natarajan, 2002). In terms of increased growth and productivity, panchagavya spray was reported to be more effective on all crops than the prescribed nutrients and growth (RFS).

CONCLUSIONS

Considering the growth and yield performances of radish, Newly Modified Panchagavya (NMPG) with tested concentrations showed significant higher values compared to other organic applications. Further, 4% of NMPG solution can be recommended for obtaining the better growth and yield of *Raphanus sativus* as a eco-friendly cultivation strategy with optimum production.

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ISOLATION AND CHARACTERIZATION OF YEASTS FROM LOCALLY AVAILABLE FOODS

Chandimala U.R.[1], Rajawardhana D.U.[2]*, Liyanage P.L.N.[3] and Hewajulige I.G.N.[2]

[1] Institute for Agro-Technology & Rural Sciences, University of Colombo, Weligattha New Town, Hambantota, Sri Lanka
[2]Industrial Technology Institute, Bauddhaloka Mawatha, Colombo-07, Sri Lanka
[3]Department of Food Science and Technology, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka

*Corresponding Author: upekarajawardana@yahoo.com (https://orcid.org/0000-0003-0425-1707)
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ABSTRACT

A study was conducted to identify different isolates of yeasts which are prospective to be utilized in various industries from locally available foods. Altogether 24 yeast isolates were obtained from fermented fruits and vegetables (banana, cabbage, grapes, lime and mango), pudding, bee honey, toddy and fermented fish samples using the pour plate method. Characterization of the yeasts using several biochemical tests (urease, catalase, liquid carbon and nitrogen assimilation and sugar fermentation tests) revealed that this pool was composed with yeast strains belong to genera of Saccharomyces, Kluvyeromyces, Candida, Pseudozyma, Cryptococcus, Rhodotorula and Debaryomyces. The most effective lactose fermenters were identified as viable candidates for bioethanol production and for manufacturing fermented dairy products for lactose intolerant people. Yeasts with the highest biomass production were suggested as the best viable candidates for industrial single cell protein production using whey, the major byproduct of the dairy industry. The five thermo-tolerant yeasts (Y55, Y57, Y58, Y59 and Y70) and Y069 which was optimally active under 10C were recognized as suitable for industrial applications. The isolates tolerant for high osmotic pressure conditions were identified as potential isolates to be used in highly concentrated food products. Sugarcane juice was recognized as a possible medium for the cultivation of these yeasts in industrial settings. The beneficial yeasts forecasting in this study are expected to screen using molecular biological methods to utilize them in industrial applications.

Keywords: Characterization, Fermented foods, Isolation, SCP, Yeasts

INTRODUCTION

Yeasts are single celled eukaryotic organisms belonging mainly to the *Ascomycetes*, classified under kingdom Fungi. So far the total number of yeast species discovered is around 1500. Even though yeasts are found in many diverse environments, yeast species have highly specialized natural habitats and thus, it is possible to isolate specific strains from appropriate habitats. (Chandrasena *et al*., 2006). Studies conducted worldwide have revealed that many beneficial yeast strains are possible to be isolated from various fermented foods (Dubash *et al*., 2010; Gana *et al*.; 2014; Moreira *et al*., 2001; Obasi *et al* 2014). But, so far only a limited number of research studies have been carried out in Sri Lanka to isolate yeasts from locally fermented foods and to screen them for their beneficial properties in a broad way. Thus in this research study, the utmost attempt was to isolate maximum possible number of yeast types from a wide range of locally available foods and to identify their possible beneficial characteristics and industrial applications with the purpose of solving the most common burdens of several industries in Sri Lanka. For the identification of yeast, several biochemical tests were used along with the information available in previous literature and this study is expected to proceed with further molecular biological studies.

METHODOLOGY

Isolation and maintenance of yeast isolates

Fruits and vegetables (Grapes, Cavendish and Embul varieties of banana, mango, lime and cabbage), smoked fish, bee honey, toddy, yoghurt and pudding samples were collected from local market, Colombo, Sri Lanka for the isolation of yeast. Ten grams of each of the fruit and vegetable samples were stored in sterile stomacher bags under 25◦C allowing for fermentation. All samples were serially diluted ($10^{-1}$to 10^{-8}) with 0.85%Nacl solution. Yeasts in above dilutions were isolated and enumerated using pour plate technique. Yeast Potato Dextrose (YPD) medium was used for growing yeast. Incubation was done for 1-2 days at 25◦C. Maintenance of cultures was done following the method given by Obasi *et al* (2014) with some modifications. The discrete isolated colonies were purified by re-streaking on YPD plates and maintained on slants with 2% agar of the same medium at 5◦C in the refrigerator and as frozen stocks in liquid YPD medium with 40% glycerol at -20◦C.

Characterization of isolated yeasts

Catalase test

A small amount of colonies from each yeast isolate was transferred to clean, dry glass slides using a loop and a drop of 3% H_2O_2 placed on the slide and mixed. Rapid evolution of oxygen as evidenced by bubbling within 5-10 seconds was considered as the positive result.

Urease test

Urea hydrolysis test was conducted by preparing the urea agar base (Code: CM0053) at the laboratory as expressed by Christensen, 1946. Surface of the Urea agar slope was heavily inoculated with a pure culture of each yeast isolate to be tested. Conversion of the medium from orange to pink after storing at 35C for 3-5 hours was taken as positive reaction.

Liquid assimilation of carbon and nitrogen compounds

This was conducted by following the methodology used in the researches by Moeini *et al.*, 2004 and Nahvi and Moeini, 2004. The carbon compounds tested were sucrose, glycerol, raffinose, D-maltose, D-manitol and L-rhamnose. The nitrogen compounds tested were nitrate and L-lysine and L- Cysteine.

Sugar fermentation test

Ability to ferment sugars (lactose and sucrose) was tested following the method explained in Moeini *et al.*, 2004.

Screening for industrial applications

Ability to ferment in different temperature conditions

Fermentation of YPD broth (5 g yeast extract, 10 g peptone and 10g glucose per 1l of broth) under 10C and 45C temperature conditions was tested by using 0.04% bromocrysol purple stock solution as the indicator. Both acid and gas production during fermentation were evaluated.

Ability to ferment in sugarcane juice

Sugar cane juice of 45% distilled water on weight basis with Bromothymol blue was inoculated with yeast isolates and acid production was evaluated. Test tubes with 5ml of extract were incubated at 25C temperature for 7-10 days and color changes were recorded.

Ability to produce SCPs

Falken tubes with laboratory made whey were inoculated with actively growing yeast cultures and were stored in a shaking incubator at 25C temperature and 150rpm for 10 days. Produced protein was separated using filter method and SCP production was analyzed based on the bio mass production

Ability to grow in 50% glucose

Isolated yeast strains were inoculated in YPD medium with 50% glucose using single streaking method. After the incubation for 3-4 days their growth was analyzed.

RESULTS AND DISCUSSION

A total of 24 yeast isolates were obtained and identified according to their colony morphologies (Figure 1) and light microscopic views from the samples of locally abundant fermented food items.

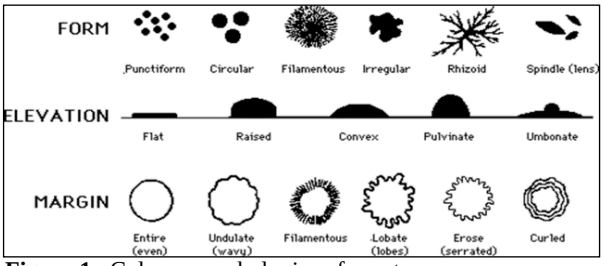

Figure 1 : Colony morphologies of yeast
Source: http://www.pinterest.com

For the isolation of yeast, peel was also used along with the pulp in all fruits and vegetables. Because, yeast prefers aerobic conditions for their growth. Being highly oxidative, peel of fruits and vegetables was identified as a good source to isolate yeast micro flora. Amongst the fermented food sources used for isolation of yeast, fermented grapes and banana have given the highest number of yeast isolates (Table 1).

Table 1 : Yeasts isolated from fermented foods

Food item	Number of yeast isolates
Fermented grapes	4
Fermented mango	2
Fermented cabbage	1
Fermented lime	3
Fermented banana(variety Cavendish)	4
Fermented Banana(variety Embul)	4
Fermented Fish	1
Bee-honey	1
Toddy	2
Pudding	2
Yoghurt	0

Though several yoghurt samples from different brand names were used in this research study for the isolation of yeast, all the attempts became unsuccessful (Table 1). A scientific study related to the isolation of yeast from yoghurts in Brazil, conducted by Moreira *et al.*, 2001 states that the improper storage conditions lead to higher

contaminations of yoghurt with yeast. Also he provides a clue that a systematic contamination at the source is also a possible reason even though it was not a reason for the observations in that study. Based on this information, it can be resolved, that yeast isolation failed from the selected yoghurt samples due to the absence of the above mentioned contaminations in the production process. Moreover, the addition of chemicals such as H_2O_2 for the raw milk before processing to extend the keeping quality, practiced by bulk yoghurt producers may be a possible reason for this.

The morphological characteristics of the colonies of isolated yeasts included spherical, creamy smooth, flat types, some with pinkish or yellowish colors, and some with wavy edges. It was especially noticed that all the three yeast isolates obtained from fermented lime samples and the two isolates from mango samples were very creamy compared with others and thus they were predicted to possess beneficial characteristics based on the results of previous studies. Literature provides evidence that yeasts are vastly variable in cell shape, size and colony colors and pinkish to yellowish colony colors are observed due to the accumulation of carotenoids in cells (Libkind, 2012). Rij 1984 has stated that the shape of vegetative cells of yeast is worthwhile as a taxonomic criterion since their form is closely connected with the way in which they are made and in many of the situations the cell shape may be very distinctive that it may be used in generic differentiation.

Characterization of Isolated Yeast Strains

All the yeast isolates gave positive results for the catalase test, showing that all of them can produce catalase enzyme in their cells. Catalase is an enzyme produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2. Based on this observation, it was verified that all the isolates are either aerobes or facultative aerobes.

Four out of the 24 yeast isolates gave positive results for the urease test, by indicating a color change from orange to pink. It reveals that 11% of this yeast pool produces urease enzyme which split urea to form ammonia. Yeasts are capable to utilize various nitrogen sources. The ability or inability to utilize nitrate nitrogen is considered to be a valuable diagnostic criterion for decisive purposes. Many genera are characterized by their inability to utilize nitrates, e.g. *Saccharomyces, Kluyveromyces, Pichia* and *Debaryomyces* while in other genera e.g. *Hansenula* all species utilize nitrate. And there are some other genera in which both nitrate positive and negative species occur e. g. *Candida* and *Trichosporon*. All yeasts are basically capable to utilize urea in low concentrations, as the only source of nitrogen when adequate amounts of vitamins are provided and when yeasts are in a media having an organic nitrogen source such as peptone, their ability to hydrolyze high concentrations of urea in that media exhibit some variations. Urea is hydrolyzed by some sporogenous and asporogenous species and ascogenous species are deficient in hydrolyzing urea whereas it is especially marked in the genera *Cryptococcus* and *Rhodotorula* (Rij 1984). As literature reveals, urease positive yeast species have been identified as pathogenically active ones such as *Candida albicans* which cause human diseases. Thus, above urease positive four yeasts were predicted to be pathogenically important ones found in this study.

According to Ebabhi *et al* 2013, yeasts gain carbon typically from hexose sugars, such as glucose and fructose or disaccharides like sucrose and maltose while some species can also metabolize pentose sugars like xylose, alcohols and organic acids. The development of yeast in a carbohydrate source indicates that it can either ferment or utilize it by respiration. These two terms are somewhat confusing since actual assimilation by the cell takes place both during the fermentative and oxidative dissimilatory processes. Literature reveals that assimilation tests are more sensitive than fermentation tests related with discovering the occurrence of enzyme systems since they do not solely depend on the utilization of fermentable sugars and compromise the use of several carbon compounds (Rij, 1984). In this study, Y71 and Y72 were the two isolates that couldn't assimilate D-sucrose suggesting the possibility to be either a *Candida spp* or *Geotrichum capitatum* as observed by previous studies (Obasi *et al*., 2014). They were obtained from fermented mango samples. Only Y57 and Y66 have indicated inability to assimilate D-raffinose. In a study by Obasi *et al.* (2014), *Candida spp, Geotrichum spp, Rhodotorula* and *Kodamaea spp* have exhibited inability to assimilate D-raffinose. Glycerol was not assimilated only by Y74 isolate. According to the studies by Obasi *et al*., 2014, some *Candida* species and *Geotrichum* species were unable to assimilate glycerol. Ability of all the other isolates to assimilate glycerol indicates that these strains possess the glycerol kinase gene (GuT1) and a gene for mitochondrial glycerol 3-phosphate dehydrogenase (GuT2) responsible for glycerol assimilation during fermentative growth. (Obasi *et al*., 2014). D-maltose couldn't be assimilated only by Y63, Y64 and Y74 yeast isolates. In the research study by Gana *et al.* (2014), *Pseudozyma* species were for not being able to assimilate D-maltose. "The ability of the isolates to also ferment maltose shows that they possess uptake mechanism that involves two systems; an energy-dependent maltose permease (ATP to ADP) which transports the maltose intact across the cellular membrane and a maltase (alpha- glucosidase) which hydrolyses maltose internally to yield two glucose units"(Obasi *et al*., 2014).

Eight isolates (Y55, Y56, Y58, Y59, Y63, Y64, Y65 and Y70) were unable to indicate a color change in the medium indicating inability to ferment sucrose. In the research study conducted by Gana *et al*, (2014), all the isolates of *Pseudozyma* species were unable to ferment sucrose, and thus based on this information here these eight isolates can be suggested as belonging to the genera *Pseudozyma.* All the isolates except Y068, Y069, Y073 and Y075 gave positive results for the lactose fermentation test converting the green color of the YPD broth into yellow color, indicating the development of acidity in the media. But only some isolates were able to produce gas during the test exhibiting that they are highly effective in lactose fermentation. Those were the isolates obtained from grapes, banana, mango and honey. The enzyme lactase, β−galactosidase which hydrolyze lactose into glucose and galactose, is produced by many microorganisms that utilize lactose as an energy source. *Kluyveromyces* species exhibit higher activity of this enzyme than other species (Moeini *et al*., 2004). Literature reveals that *S. cerevisiae* is unable to grow and ferment in lactose medium when it is provided as the sole carbon source as it doesn't possess a lactose metabolizing system. ((Moeini *et al*., 2004; Domingues *et al*., 2010). According to Domingues *et al.* (2010), *S. cerevisiae* has majorly adapted to utilize glucose whereas *K. lactis* has adapted to lactose. Therefore, these two yeasts possess different modes of regulation which are responsible for their overall response for carbon sources and this may be the cause for major physiological differences they exhibit. Some *Debaryomyces* species, *Candida* species, *Schizosaccharomyces pombe* and *Mrakia frigida* also provide negative results for the lactose fermentation test (Moeini *et al*., 2004). In the studied yeast pool, 17%, which were lactose non-fermentative, can be predicted to be among these yeast species. In the tested yeast pool, 33%of isolates were identified to be the most effective lactose fermenters as they produced both acid and gas in the experiment. These yeasts were chosen as the best viable candidates for industrial applications related with lactose fermentation (Figure 2).

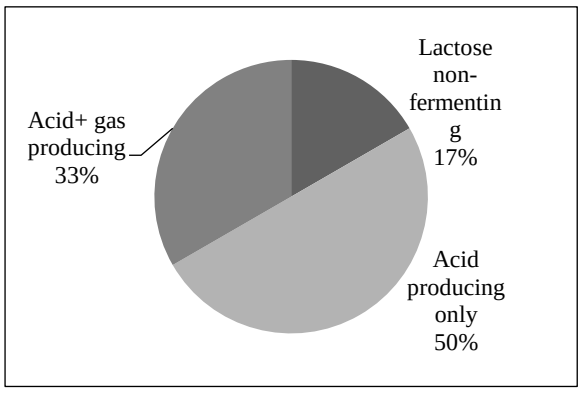

Figure 2 : Effectiveness of the lactose fermentation test

Whey, which is considered as the major waste material in dairy industry can be used as a raw material for the production of ethanol with the use of lactose fermenting yeasts. Thereby, the waste material which causes lot of environmental problems due to its disposal can be converted in to a resource that generates economic benefits. Also ethanol produced from whey through lactose fermentation can be used in products such as potable spirits printing inks and white vinegar. Lactose fermenting yeasts can be incorporated in dairy products for lactose intolerant people.

Based on the above characterization tests and colony morphologies, it was summarized that the tested yeast pool was composed with species belong to genera of *Pseudozyma*, *Cryptococcus*, *Candida*, *Rhodotorula*, *Kluyveromyces*, *Saccaromyces* and *Debaryomyces.* Molecular studies revealed that Y071, isolated from mango to be *Starmerella bombicola* which is an industrially important species due to its ability of producing large amounts (400 g/L) of sophorolipids which are carbohydrate-based, amphiphilic biosurfactants. Due to the biodegradability, low eco-toxicity and the production on renewable-resource substrates, bio-surfactants are beneficial over the chemically synthesized counterparts.

Screening for Industrial Applications

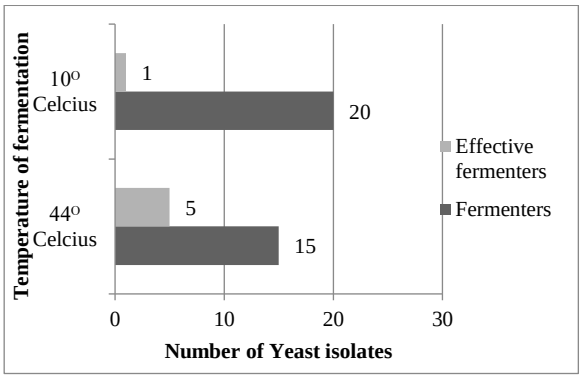

Figure 3 : Effectiveness of the fermentation process under high and low temperatures.

*Isolates which result only in color change are labelled as 'fermenters'. Isolates which result in both gas assembly and color change are labelled as 'Effective fermenters'.

Except four isolates, all others indicated the ability to ferment under low temperature conditions, at 10_oC as provided here (Figure 3). However, Y69 isolated from banana was the most effective at this temperature because gas assembly was also indicated in addition to the color change in the medium. Some products such as cheese and white wine prefer to conduct fermentation under low temperature conditions to achieve the best product properties. Y069 isolate can be suggested as the best viable candidate for such applications. Same observation was recorded from Y55, Y57, Y58, Y59 and Y70 isolates under high

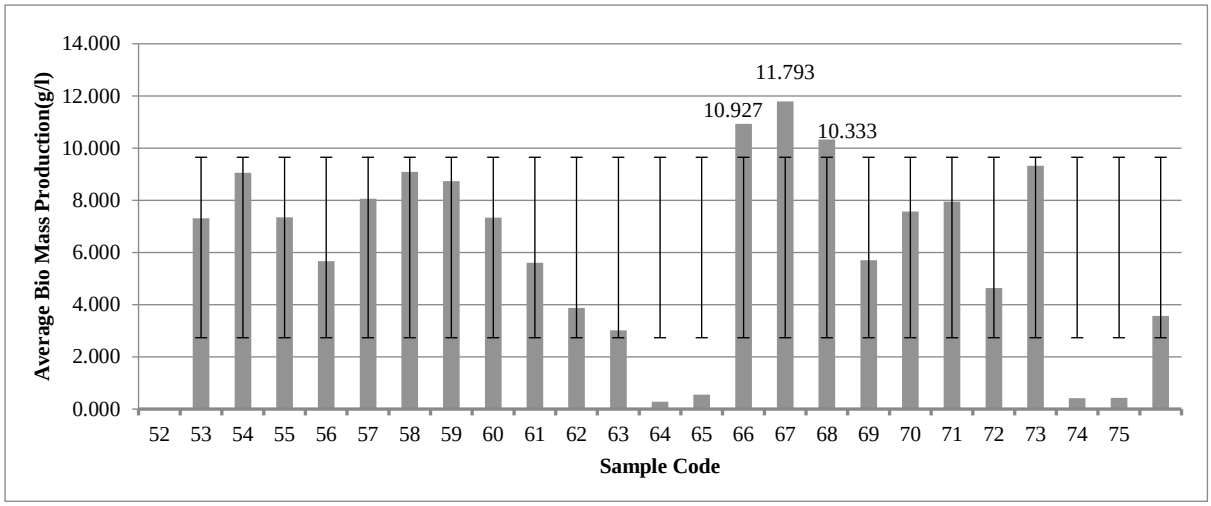

Figure 4 : Average biomass production of the yeast isolates

temperature condition.These thermo tolerant yeast isolates were obtained from fermented banana and grapes samples. Thermo tolerant yeasts can be cultured under conditions where other micro-organisms cannot grow, thus reduces the risk of contamination. The enzymes produced by such strains are also probably thermo tolerant and they are more beneficial due to their eukaryotic nature over the enzymes from bacteria (Takashima *et al*., 2009). Moreover, in tropical and subtropical environments, maintenance of low temperature in fermenters requires cooling systems which generates high production costs especially in sugarcane based fermentations. Thus, use of thermo tolerant yeasts is a good solution to overcome this problem. Based on this study, the five isolates identified as thermo tolerant can be predicted as potential candidates for such applications.

All the yeast isolates indicated color change from green to yellow suggesting that sugarcane juice is a suitable media for the cultivation of them. Sugarcane juice can be used to replace expensive media used under laboratory conditions. But as sugar refining and molasses based distilleries are the major industries in Sri Lanka, using molasses which is produced as co-products of sugar production, to generate ethyl-alcohol is much more economically viable (Chandrasena *et al*., 2006). Thus, screening of yeast isolates that are capable to grow in sugarcane juice must be further screened for their ability to grow in molasses to make it more economically viable in industrial applications.

Average biomass production among yeast isolates when introduced to whey media, exhibited a much variation within the range of 0.280 to 11.793 g/L (Figure 4). Calculated mean value of the data series was 6.180g/L. Based on the results, banana and pudding were the food sources from which the best three biomass producers were obtained. Average bio mass production was highest in Y66 yeast isolate obtained from a pudding sample and it was 11.7933 g/L in amount (Figure 4). A similar bio mass production has been reported in the research study conducted by Moeini *et al*., 2004 and the respected yeast was *K. lactis*. In addition to that, this literature reveals that the bio mass production can be raised further more by using mixed cultures of yeast species such as *K. lactis, K.marxianus* with *S. cerevisiae* and by the supplementation of the medium with a nitrogen source like ammonium sulphate.

Yeast isolates that have exhibited satisfactory level of biomass production have the potential to be utilized in food and fodder yeast production in a successful way. The SCP production using abundant raw-materials is one of the highly demanded applications of yeast micro flora in modern world. There are several benefits of producing yeast based SCPs using whey. The first thing is, whey being the major by-product in dairy manufacturing firms, it causes huge disposal problems due to the high organic matter content. Secondly, whey which contains proteins is not successfully utilized in producing another important product in Sri Lanka. In addition to that, SCPs have a huge demand in current food industries as it has been identified as a good solution to fulfill the dietary protein requirement in increasing populations, especially for vegetarians. Also, SCPs can be used to feed farm animals as a rich source of protein.

In 50% glucose media, only Y066 isolate indicated a growth negative response and it was only a 4% from the total yeast pool. Therefore, 96% of the yeast pool was predicted to thrive well under high osmotic pressure conditions. Thus, they were recognized as suitable yeasts to use in high concentrated food products. Ability to grow at 50% glucose has also been tested in some previous studies as a physiological test to identify yeast strains. According to the research by Gana *et al.* (2014),

Pseudozyma species and *Cryptococcus aerius* have exhibited inability to grow under these conditions.

CONCLUSIONS

There are several foods (fruits and vegetables, fish, bee-honey, toddy and pudding), which are highly locally available could be considered potential sources for the isolation of a variety of yeasts. The isolated pool of yeast was composed of with creamy, circular or irregular, flat, entire, undulate and curled colonies while some of them were pigmented with yellow or pink color compounds. The characterization tests and colony morphology revealed that this pool of yeast belong to genera of *Pseudozyma, Cryptococcus, Candida*, *Rhodotorula*, *Kluyveromyces*, *Saccaromyces* and *Debaryomyces.* Different strains of yeast isolates were identified as viable candidates for utilizing in industrial applications; SCP production from whey, bio ethanol production from lactose containing media, i.e. whey, manufacturing dairy products for lactose intolerant people, fermentations under low or high temperature conditions and production of fermented foods with high concentrations of sugar. Sugarcane juice was recognized as an inexpensive media for industrial cultivation of all the isolated yeasts..

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VERMICOMPOST – A BETTER ORGANIC MANURE IN INTEGRATED PLANT NUTRIENT SYSTEM FOR *Vigna radiata* (MUNG BEAN)

Jeyavaran A[1], Bandusekra B.S.[1*], Sivakumar K.[2] and Gunathilake C.[1]

[1]Institute for Agro-Technology and Rural Sciences, Weligatta-New Town, Hambantota, Sri Lanka
[2]Sri Lanka School of Agriculture, Vavuniya

**Corresponding Author: supun@uciars.cmb.ac.lk (https://orcid.org/0000-0002-6382-1071)*
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ABSTRACT

The mung bean (Vigna radiata L.) is a member of the Fabaceae family. It is a major grain legume widely farmed in tropical and subtropical regions worldwide. Mung bean is a versatile crop that responds well to fertilizers and low nitrogen requirements due to its leguminous nature. The present experiment evaluated the effects of different types of organic manure on mung bean growth, yield, and physicochemical properties to identify the best integrated plant nutrient management system. There were five treatments; T_1 application of inorganic fertilizer (DOAR), T_2– application of compost (10 mt/ha) and ¼ DOAR, T_3 – application of vermicompost (8 mt/ha) + ¼ DOAR, T_4 – application of cow dung (4 mt/ha) + ¼ DOAR and T_5 – no application of any fertilizers. Mung bean variety MI-06 was used as genetic material (raw seeding), and organic manure was added at the land preparation, and synthetic fertilizer was added as a split application. The variables such as plant height, number of pods per plant, seed yield, thousand seed weight, moisture content, protein content, and bulk density of the flour were varied among treatments. It was found that there were significant differences among treatments on all parameters except plant height and pH of the flour.. The highest number of seeds per pod, thousand seed weights, and total grain yield were recorded from vermicompost, which was not significantly different from inorganic fertilizer. Significantly lowest bulk density of flour was recorded from inorganic fertilizer, whereas others did not show a statistical difference. Interestingly, the significantly highest protein content was recorded from vermicompost, followed by the inorganic fertilizer. The present study suggested that applying a vermicompos to the integrated plant nutrient mixture could increase the quality and quantity of mung beans, especially yield and protein content.

Keywords: Mung bean, Physiochemical properties, Vermicompost

INTRODUCTION

Mung bean (*Vigna radiata L.*), also known as, green gram, is one of the most important pulse crops and an excellent source of high-quality protein (Patel et al., 2018; Mahalingam et al., 2018). It consists of about 25 % protein, almost 2.5-3.0 times more than cereals. Sprouted mung bean whole seed is used in South India for preparing curry or a savoury dish. India is the largest producer and consumer of pulses in the world. India primarily produces Bengal gram (chickpea), red gram (tur), lentil (Masur), mung bean (green gram), and black gram (urad). Pulses are the major source of protein for vegetarians, and crop residues are a major source of high-quality livestock feed. Mung bean has the edge over other pulses because of its high nutritive value, digestibility, and non-flatulent behaviour. It is grown principally for protein-rich edible seeds, which contain 24% crude protein, 56.7% carbohydrates, 1.3% fats, 3.5% minerals, 0.43% lysine, 0.1% methionine and 0.04% tryptophan (Kachroo, 1970).

Mung bean is one of the most important grain legumes in the traditional farming systems of Sri Lanka. It is one of the principal but cheapest protein sources and its importance as a component of the Sri Lankan diet has grown over the years. Mung bean contains a high percentage of easily digestible protein, and its essential amino acid composition is also complementary to the staple food, rice. In addition to being an essential source of human food and animal feed, mung bean also plays an important role in sustaining soil fertility by improving physical properties and fixing atmospheric nitrogen in the soil (Kumara et al., 2021).

The local production of mung beans has declined over the last two decades, and 49.8% of the total mung bean requirement is still being imported (Department of Customs, 2019). In this context, it is clear that increasing the production of mung beans is a must to achieve the government's target. However, in Sri Lanka, there is a large gap between the actual yield and the potential yield of mung bean due to various issues—i.e., unavailability of nutrient-rich soil, lack of quality seeds, unsuitable climatic factors, traditional cultivating practices, improper weed

management, etc. Yield loss due to weed competition can significantly hinder achieving potential yield in most crops, including mung bean (Kumara et al., 2021).

Organic farming preserves the ecosystem. Symbiotic life forms are cultured ensuring weed and pest control and optimum soil biological activity, maintaining fertility. Organic farming neither demands synthetic fertilizers nor harmful chemicals (pesticides & fungicides) for controlling weeds, insects and pests. Synthetic fertilizers only are harmful to soil and the aerial environment because the inorganic fertilizers mainly contain major nutrients NPK in large quantities and neglect the use of organic manures and bio-fertilizers; hence have paved the way for deterioration of soil health and, in turn, ill-effects on plants, human being and livestock (Choudhry, 2005).

Using organic manures alone as a substitute for chemical fertilizers is not profitable and will not be adequate to produce the potential yields of of high-yielding varieties. The use of organic manures and inorganic fertilizers leads to increased productivity and sustains soil health for a more extended period (Alley et al., 2009). Although not useful as sole sources of nutrients, organic manures act as good complementary nutrient sources with inorganic fertilizers (Chaudhary et al., 2004), which have carried over to succeeding crops. Using organic manures alone or combined with chemical fertilizers will help improve the soils' physicochemical properties and efficiently use applied fertilizers to improve seed quality and quantity. Organic manures provide a suitable substrate for the growth of microorganisms and maintain a favourable nutritional balance and soil physical properties. It is recognized that combined sources of organic matter and chemical fertilizers play a key role in increasing soil productivity. Mung bean yield and quality can be improved by the balanced use of fertilizers and by properly managing the organic manures.

Currently, Sri Lankan agriculture policy focuses on minimizing synthetic fertilizer usage as much as possible, especially for field crops. The present research addressed such an objective under low synthetic fertilizer application with integrated plant nutrient management systems (IPNS). In this context, farmers have to face some difficulties in finding the correct type of organic manure in appropriate quantities to adopt the IPNS systems for mung bean cultivation. Considering the above facts, the present investigation was carried out to determine the effect of three types of organic manures and inorganic fertilizers in an integrated manner on the yield and physiochemical properties of the mung bean.

METHODOLOGY

Experimental site

The experiment was conducted in the field at Sri Lanka School of Agriculture, Vavuniya, and the study was conducted from September 2021 to February 2022.

Experimental procedure

Mung bean MI-06 was used as the genetic material of the study, and practised raw seeding. All the agronomic practices were carried out as recommended by the Department of Agriculture, Sri Lanka, except for fertilization. The field experiment was laid out in Randomized Complete Block design (RCBD) with five treatments and four replications. Each plot size was 2 m^2 and separated each other 1.0 m space within the blocks. Each plot contained 16 plants as experimental units. The experimental site was divided into four blocks based on the slope of the land. Each treatment was randomized for every block as four replicates. Different treatments and its description are mentioned in table 1.

Table 1: The treatment combination of the experiment

Treatment	Treatment Description
T1- IPNS Compost	Application of compost at 10 MT/ha and Urea – 8.75 kg/ha, TSP – 25 kg/ha and MOP – 18.75 kg/ha
T2 – DOAR	Application of Urea – 35 kg/ha, TSP – 100 kg/ha and MOP 7. kg/ha
T3- IPNS Vermicompost	Application of vermicompost at 8 MT/ha and Urea – 8.75 kg/ha, TSP – 25 kg/ha and MOP – 18.75 kg/ha
T4- IPNS Cow dung	Application of cow dung at 4 MT/ha and Urea – 8.75 kg/ha, TSP – 25 kg/ha and MOP – 18.75 kg/ha
T5- Control	Without any external fertilization

The compost rate was selected based on the DOAR. Cow dung and vermicompost rates were selected based on the N% (Alidadi et al., 2014), which was proportionate to maintain similar N% availability.

Data collection

Data were collected mainly on two aspects as growth/yield parameters and postharvest parameters. Growth and yield parameters include plant height, number of pods and grain yield. Postharvest parameters include both the physical and chemical properties of grains.

Growth and yield parameters

Plant height was measured using a meter ruler and recorded in centimetres. The plant height was taken from ground level to the tip of the shoot. The number of pods was counted per treatment.

Fully matured pods were harvested from 8 plants of each plot to avoid the border effect. Then grains were

separated from the plant, and the dry weight of the grains was measured. All the weight measurements were taken using an analytical balance (0.1 grams).

Physical properties

Moisture content

Seed moisture content (SMC) is the amount of water in a seed. SMC is expressed in terms of the weight of water contained in the seed as a percentage of the total weight of the seed before drying, known as the wet-weight (wb) or fresh-weight basis (International Seed-Testing Association [ISTA] 2005).

$$SMC\ (\%\ wb) = \left\{\frac{wet\ weight - dry\ weight}{wet\ weight}\right\} \times 100$$

Weight

The fresh weight of 20 randomly selected seeds was recorded, and the mean values were computed. Dry weight: 20 randomly selected seeds were kept in a hot air oven at 105 °C overnight and their dry weight was recorded using electric balance. And the mean values were recorded.

1000 seeds weight

Collected seeds from the pods were counted and took the weight of the seeds and that weight was calculated for 100 seeds.

$$1000\ seeds\ \ weight = \{(weight\ of\ X\ number\ of\ seeds) \div X\} \times 1000$$

Number of seeds per pod

Seeds grow in groups, nestled within a pod or a several-seeded dehiscent fruit called a pod, and the number of seeds in the pods was collected and counted manually.

Bulk density

The bulk density of a powder is determined by measuring the volume of a known mass of powder sample, which may have been passed through a sieve into a graduated cylinder.

Pass a quantity of powder sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated cylinder of 250 mL (readable to 2 mL), gently introduce, without compacting, approximately 100 g of the test sample (m) weighed with 0.1 percent accuracy. Carefully level the powder without compacting, and read the apparent unsettled volume (V_0) to the nearest graduated unit. Calculate the bulk density in g per mL by the formula. Generally, replicate determinations are desirable for the determination of this property. If the powder density is too low or too high, such that the test sample has an apparent untapped volume of either more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as a test sample, such that its apparent untapped volume is 150 mL to 250 mL (apparent volume greater than or equal to 60 percent of the total volume of the cylinder); the mass of the test sample is specified in the expression of results. For test samples having an apparent volume between 50 mL and 100 mL, a 100 mL cylinder readable to 1 mL can be used; the volume of the cylinder is specified in the expression of results.

Chemical properties

Proteins

30 mg of samples were weighed into Kjeldhal digestion flasks. 1.3g of K_2SO_4 and 40mg of HgO were added as a catalyst mixture, followed by 25 ml of concentrated H_2SO_4. The samples (in 27 Kjeldhal digestion flasks) were left to digest in a heating digester for 4 hours after connecting to the fume trap and attached to the pump. Flasks were left to cool for one hour.

Samples were dissolved in the minimum amount of ammonia-free distilled water and transferred to a semi-micro Kjeldhal distillation apparatus that had been previously conditioned by slowly passing steam for several minutes. 8 ml of NaOH solution was added to the flask. The indicator was prepared by mixing 2 mL of methyl red solution with 1 ml of methylene blue solution. 5ml of 4% Boric acid solution and three drops of the indicator were taken into a titration flask and kept at the end of the digestion apparatus to trap the ammonia. Steam was passed through the flask until about 20ml of distillate was recovered.

All distillates were titrated with the standard HCl solution (0.02M HCl). Three replicates were done. Plate 3.4 shows the colour changes (green) during distillation, and plate 3.5 shows the colour changes (from green to purple) after being titrated with HCl solution. Protein percentage was calculated using the following equation protein (%) = Nitrogen percentage x 5.70 (Source: Modification of AOAC official method 920.87)

$$\text{Nitrogen (\%)} = \frac{(\text{Sample tite-Blank titer}) \times \text{Molarity of HCl} * 14}{\text{Weight of the sample}} \times 100\text{N (\%)}$$

PH

The pH was measured by making a 10% (w/v) flour suspension of each sample in distilled water. Each sample was then mixed thoroughly in a plastic beaker, and the pH was recorded with an electronic pH meter.

Data Analysis

Collected data were analyzed using analysis of variance (ANOVA) procedures by using the statistical package Minitab 18 version. The difference between the treatments was compared using Duncan's multiple range test (DMRT) at a 5% significance level.

RESULTS AND DISCUSSION

Vegetative parameters

The results showed no significant differences between the treatments on plant height during the entire growth period. The plant height at 30 days after sowing (DAS) differed significantly due to the combined application of organic and inorganic fertilizers. It was revealed that there were significant differences between the treatments on pod number. The highest and similar average number of pods were recorded from compost, vermicompost and DOAR (12 pods), whereas the lowest was observed from cow dung (11.7) and control (12.2), respectively (Table 2).

The number of pods per plant of Mungbam is influenced by organic and inorganic fertilizers, as reported by Armin et al. (2016). Because of the diverse combinations of organic and inorganic fertilizer doses, the number of pods per plant varied significantly. A combination of vermicompost and inorganic fertilizers produced the highest number of pods per plant. When organic and inorganic fertilizers were used together, the number of pods per plant grew more than when inorganic fertilizer was used alone. This could be resulted by combining organic and inorganic fertilizers enhances soil's physical qualities, resulting in improved soil health and nutrient utilization efficiency.

Higher gain yield in mung bean was obtained in the treatments where vermicompost (312.5 ± 24.9 g) and was not significantly different from DOAR (300.0 ± 18.1 g) (Table 2). Both treatment compost and cow dung had similar grain yields of 262.5 g, which were not significantly different from DOAR (Table 2). Armin *et al.* (2016) mentioned that the seed yield of mung bean per plant showed significant variation due to the different combinations of organic and inorganic fertilizer doses. That study showed significant performance from vermicompost-based integrated fertilization compared to poultry manure-based integrated fertilization. Furthermore, Patil (1998) reported that in groundnut, the maximum seed yield plant^{-1} was recorded with the application of vermicompost at 2.50 t per ha + inorganic fertilizer, the application of inorganic fertilizer alone. The results of the present study and literature confirm that combining vermicompost and inorganic fertilizers increased the seed yield of plant-1 than using inorganic fertilizers alone.

Organic fertilizers aid in increasing the organic matter content of the soil, lowering bulk density and minimizing compaction. Plants are provided with an appropriate growing environment, allowing for improved growth and development. Many scientists discovered similar results while trying various crops. Channaveerswami (2005) observed that a combination of organic and inorganic fertilizers performed better in groundnut, while Rajkhowa et al. (2002) indicated that inorganic fertilizers performed better in mung beans.

Physiochemical parameters

The study showed a significant difference in all the tested physiochemical properties against different treatments (Table 2). The 100 seed weight varied from 45.2 g to 52.0 g among treatments. The higher values in thousand seed weights were obtained in the treatment DOAR as 52.0 g, but that does not significantly different from the vermicompost (52,7 g). Cow dung, compost and control did not significantly differ in 1000 seed weight (Table 3). Karpagam and Rajesh (2014) mentioned that 1000 seed weight is mainly affected by grain shape, grain size and the filling of kernels. In this regard, grain size and shape are similar since the same variety was used. The possible reason for having variation in 1000 seed weights could be the N utilization and availability. Significantly lowest moisture content was recorded from compost (9.8 %) treatment, whereas it was highest in cow dung (12.32 %). The samples' bulk density varied between 0.55 to 0.66 g/cm^3. Significantly lowest bulk density was recorded from the treatment DOAR. All the other treatments did not significantly differ in bulk density (Table 3).

Amir et al. (2016) found that organic and inorganic fertilizers influenced data on mungbean's 1000-seed weight (g) in a Mungbam experiment. The diverse combinations of organic and inorganic fertilizer doses resulted in significant variance in 1000-seed weight. The results show that using organic and inorganic fertilizers raised the 1000-seed weight more than using only inorganic fertilizers. This could result from IPNS systems, which include plant nutrients, growth-promoting chemicals, and beneficial microorganisms, which provide favourable soil conditions that improve nutrient utilization efficiency when combined with inorganic fertilizers.

The protein content of the treatments varied from 20.84 to 26 28 g/100g (Table 3) The highest value for protein content was recorded from vermicompost followed by cow dung (25.40 g/100g), DOAR (22.91 g/100g), compost (22.42 g/100g) and control. The pH value did not change much among the treatments, ranging from 6.38 to 6.61 (Table 3).

Overall, the integrated nutrient management treatment with vermicompost had a high 1000 seed weight, bulk density and protein content competitive with only inorganic fertilizer treatment (Table 3). And further yield and the number of pods also had a similar observation (Table 2).

Table 2: Effect of integrated nutrient management on plant height, pod number and yield attributes of mung bean

Treatment	Plant Height (cm)				Pod Number	Grain Yield (g) (MT/ha)
	2nd week	4th week	6th week	8th week		
IPNS Compost	11.9 ± 1.1 [a]	14.6±1.6 [a]	17.7±1.5 [a]	18.2±1.3 [a]	12.0 ± 0.0 [a]	262.5 ± 17.5[bc] (1.32 MT/Ha)
DOAR	10.4± 0.7 [a]	13.6±0.6 [a]	16.4±0.9 [a]	18.1±0.7 [a]	12.0 ± 0.0 [a]	300.0 ± 18.1 [ab] (1.50 MT/Ha)
IPNS Vermicompost	11.1±0.9 [a]	15.0±1.3 [a]	18.5± 1.1[a]	19.7±0.4 [a]	12.0±0.0 [a]	312.5 ± 24.9 [a] (1.56 MT/Ha)
IPNS Cow dung	10.1± 0.2 [a]	12.9±0.5 [a]	16.6±1.3 [a]	17.7±1.2 [a]	11.7± 0.2 [a]	262.5 ± 18.5 [bc] (1.32 MT/Ha)
Control	9.5± 0.4 [a]	12.1±0.1 [a]	14.4±0.5 [a]	17.7±0.4 [a]	11.2± 0.2 [b]	237.5 ± 11.0 [c] (1.18 MT/Ha)

Values represent the mean ± standard error of four replicates. Means followed by the same superscripts in the same column are not significantly different at 0.05 probability level according to DMRT. Yield values in MT per hectare were mentioned within bracket.

Table 3: Effect of integrated nutrient management on physiochemical properties of mung bean grains

Treatment	Physical parameters			Chemical parameters	
	Moisture (%)	Thousand seed weight (g)	Bulk density (g/cm3)	pH	Protein content (g per 100g)
IPNS Compost	9.80 ± 0.03 [e]	46.2 ± 0.75[b]	0.66 ± 0.01[a]	6.61 ± 0.08[d]	22.42 ± 0.13[c]
DOAR	10.47 ± 0.07[c]	52.0 ± 1.23[a]	0.55 ± 0.01[d]	6.52 ± 0.06[e]	22.91 ± 0.16[c]
IPNS Vermicompost	10.27 ± 0.06 [d]	51.7 ± 1.03 [a]	0.65 ± 0.01[a]	6.39 ± 0.09[b]	26.28 ± 0.15[a]
IPNS Cow dung	12.32 ± 0.13 [a]	44.5 ± 0.29[b]	0.66 ± 0.01[a]	6.38 ± 0.06[c]	25.40 ± 0.18[b]
Control	10.90 ± 0.05 [b]	45.5 ± 0.29[b]	0.65 ± 0.01[a]	6.51 ± 0.03[a]	20.84 ± 0.17[d]

Values represent the mean ± standard error of four replicates. Means followed by the same superscripts in the same column are not significantly different at 0.05 probability level according to DMRT.

Interestingly high protein content was recorded from vermicompost treatment could result from high nutrient utilization. The two kinds' distinct responses to fertilizer treatment in terms of vegetative and physiochemical characteristics demonstrate that the fertilization method is a major factor in determining cowpea output. According to an experiment conducted by Abedi et al. (2010), nitrogen fertilizer has no significant impact on wheat seed protein content. However, compost had a substantial effect on seed protein, with the 60 MT compost ha $^{-1}$ treatment yielding the highest amount of seed protein. According to the findings, there were no significant variations in seed gluten concentration between the nitrogen and compost treatments. The results on seed water-soluble protein and gluten contents were unaffected by the N approach, according to Abedi et al. (2010).

These findings are consistent with previous researchers who discovered that N feeding has a considerable effect on the albumin-globulin amount (Dupont and Altenbach 2003; Pedersen and Jorgensen 2007;). As previously stated, wheat grain protein content is influenced by genotype, growing system, and environmental circumstances. In other words, while increasing nitrogen availability boosted all protein components significantly, the effect on grain protein varies on the cultivar seeded, due to various utilization of available soil nitrogen, particularly during stem elongation. One of the most important findings was a considerable increase in grain protein content when organic material was utilized in conjunction with nitrogen fertilizer.

The study results reveal that vermicompost-based IPNS perfume is better than the other IPNS in mung bean crops cultivated in the Jaffna area. Senarathne (2017) investigated the feasibility of producing vermicompost in low-country dry zone areas in Sri Lanka. In that study, problematic aquatic weeds were used for the preparation of compost and vermicompost. The results showed that vermicompost was superior in all the properties compared to compost in the same substrate. Further, the study revealed that aquatic weeds such as *Salvinia molesta*, *Eichhornia crassipes* and *Lagenandra toxicaria* , which are readily available in the low country dry zone, can be successfully used to produce vermicompost under the low cost of production (Senarathne 2017).

The literature confirms that vermicompost is better because it contains most macronutrients, and its nutrient composition is often greater than the regular garden soil and compost. Vermicomposting increases soil fertility and water resistance and helps in germination, plant growth, and crop yield (Olle, 2019). Vermicompost contains beneficial bacteria (102-106 per gram of vermicompost of Actinomycetes, Azotobacter, Rhizobium, Nitrobacter, and Phosphate Solubilizing Bacteria) and plant growth regulators (auxins, cytokinins, and gibberellins). (Chaulagain et al., 2017).

The present combination showed competitive performances against fully inorganic cultivation and could be replaced by the vermicompost-based IPNS system. The reason for such an observation could result from improving soil properties and increasing N fertilizer use efficiency. Moreover, which may create a healthy microenvironment in the root zone where habitat for beneficial microorganisms and support for the symbiotic relationship in root nodules. Further, the present study suggests assessing the effect of continuous application of vermicompost-based IPNS on soil properties (physical, chemical and biological) and yield and postharvest properties.

CONCLUSIONS

The present study on integrated plant nutrient system (IPNS) on mung bean has proved that it caused significant effects on pod number, grain yield, 1000-seed weight, grain pH, bulk density, moisture content and bulk density except for plant height. Based on the results, it could be concluded that the application of vermicompost-based IPNS can be replaced fully inorganic without affecting yield and physicochemical properties.

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JOURNAL ETHICS

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ATRS Journal,
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Sri Lanka.

Tel: +9447 2034200
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