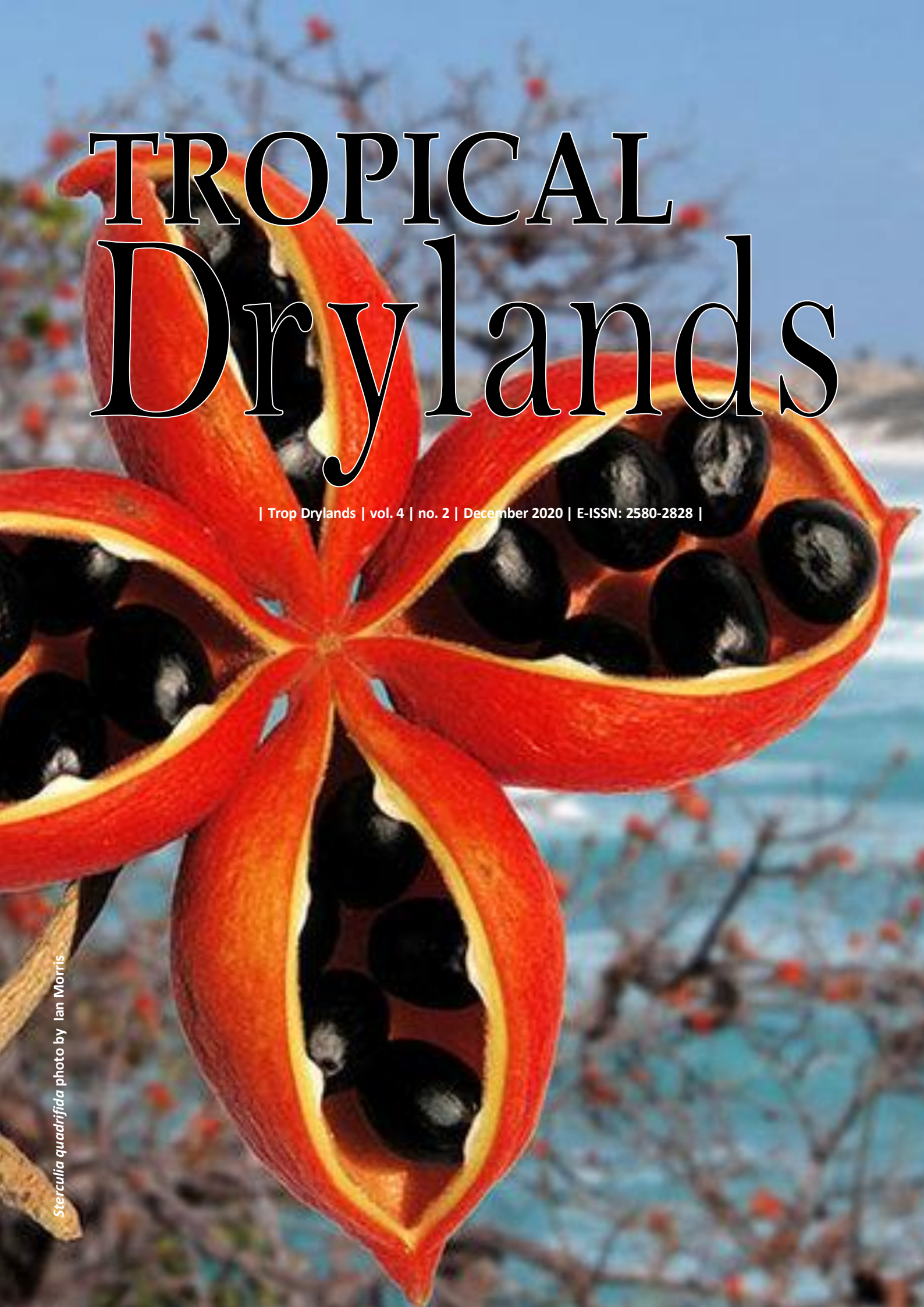


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Sterculia quadrifida photo by Ian Morris



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Effect of donor plants and rooting medium on stem cutting propagation of faloak (*Sterculia quadrifida*)

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Abstract. Rianawati H, Siswadi. 2019. Effect of donor plants and rooting medium on stem cutting propagation of faloak (*Sterculia quadrifida*). *Trop Drylands* 4: 31-35. Faloak (*Sterculia quadrifida* R.Br.) is a semiarid species and an important medicinal plant from Sterculiaceae family. This species is threatened by overexploitation and low seed production, reducing its population in the wild. Thus, planting is necessary to conserve this species, but limited knowledge exists regarding its propagation. The study was conducted to investigate the effect of donor plants and rooting medium on stem cutting propagation of *S. quadrifida*. The stem cuttings from *S. quadrifida* seedlings/juvenile (3.7-8.5 mm in diameter) and *S. quadrifida* trees/mature plants with two different size of diameter, small diameter (5.8-13.8 mm) and large diameter (15.4-24.4 mm), were planted in three various growth medium (soil, soil+manure of 1:1 ratio, and coco peat). The result showed that the survival rates of stem cuttings from juvenile donor plants and mature plants with large diameter were higher (15%-25%) than the survival rate of mature cuttings with small diameter (0%-5%). However, the rooting medium had no significant effect on the survival rate of stem cutting. Additionally, variables measured at the end of the observation, such as the number of roots/cutting, root length, number of nodes, number of leaves, leaf size were not significantly different among the survived stem cuttings from different treatments.

Keywords: Cutting, faloak, stem, *Sterculia quadrifida*

INTRODUCTION

Sterculia quadrifida R.Br., or locally called faloak, is a semi-arid species belongs to Sterculiaceae family. *S. quadrifida* can grow naturally in dry and rocky land at altitudes of up to 1000 m above sea level (Siswadi and Rianawati 2014). In East Nusa Tenggara (NTT) Province, Indonesia, *S. quadrifida* barks are used as a traditional remedy for liver diseases, gastroenteritis, diabetes, rheumatoid arthritis, cancer, typhoid, anemia, hypertension and other internal diseases (Siswadi and Rianawati 2014; Siswadi et al. 2013). Scientifically, many studies revealed that plant parts of *S. quadrifida* contain active compounds (Siswadi et al. 2013) such as antioxidant (Amin et al. 2015; Hilaria and Tarigan 2018; Lulan 2018; Saragih and Siswadi 2019), antiviral (Dean et al. 2019), antifungal (Rollando et al. 2019), antibacterial (Kapitan 2018; Syahrani et al. 2017) and anticancer (Rollando and Alfanaar 2017).

Because of its benefits, the demand for *S. quadrifida*, especially *S. quadrifida* barks, has been increasing nowadays, suggesting that *S. quadrifida* has a considerable high economic value (Siswadi et al. 2016). As a consequence, along with the increasing demand for *S. quadrifida* barks, the rate of *S. quadrifida* exploitation is also getting higher that threatens its sustainability. People tend to harvest *S. quadrifida* bark excessively without considering the regeneration ability of *S. quadrifida*. Such practices lead to a decrease in the number of *S. quadrifida* stands. Therefore, both generative and vegetative

propagation should be given more attention to conserve *S. quadrifida*.

A previous study by Siswadi et al. (2012) reported that *S. quadrifida* could be propagated by a generative method. The study also revealed that pre-treatment of scarification of *S. quadrifida* seeds is required to improve the germination rate by soaking *S. quadrifida* seeds in the water for 12 hours. The most suitable medium for *S. quadrifida* seedling to grow was the combination of black soil (grumusol), sand, and manure in a ratio of 1:1:1 (Siswadi et al. 2012). However, generative propagation of *S. quadrifida* has some obstacles because of the limited distribution and seed production of *S. quadrifida*. Even though *S. quadrifida* can grow naturally over several regions in NTT (Timor, Sumba, Alor, Flores Islands) (Siswadi et al. 2013), *S. quadrifida* trees are not eventually distributed in adequate population. Furthermore, many *S. quadrifida* trees in the population do not produce fruit/seed annually or do not flower at the same time, so it is also another obstacle for conducting generative propagation and genetic conservation as well. Thus, vegetative method could be an alternative method for propagating *S. quadrifida*. Moreover, vegetative propagation may shorten the rotation time of *S. quadrifida* as compared to generative propagation method.

Siswadi et al. (2017) studied vegetative propagation of *S. quadrifida* by stem cutting in 2016. However, the results indicated that the survival rate of stem cutting *S. quadrifida* was very low (no more than 7%). According to many

studies on vegetative propagation, the success of cuttings to produce roots is affected by many factors including, the plant parts used for cuttings, the sources of donor plants, plant age, cutting size, planting medium and environmental factors. Hence, the objective of this study was to examine the effect of donor plants and rooting medium on the survival rate of stem cutting propagation of *S. quadrifida*. This paper tests the hypotheses that the juvenile donor plants will improve the survival rate of stem cutting; and the variety of cutting media will have an impact on the rooting growth of stem cutting.

MATERIALS AND METHODS

This research was conducted from January to April 2019 in the nursery of Environment and Forestry Research Development Institute of Kupang, East Nusa Tenggara (NTT) Province, Indonesia.

Plant material source and rooting medium

Cutting materials were taken from two types of donor plants of *S. quadrifida*, namely juvenile and mature donor plants. Juvenile donor plants were collected from 3-year olds *S. quadrifida* seedlings which were maintained in the 25 cm diameter of polybag in the nursery. Whereas, mature donor plants were selected from *S. quadrifida* trees that grow naturally around Kupang regency. In the selection of donor plants, both juvenile and mature plants should be vigor and free from pests and diseases.

The diameter sizes of cuttings that are taken from mature donor plants varied, so these were divided into two different sizes; small diameter (5.8-13.8 mm) and large diameter (15.4-24.4 mm). The range of diameter sizes of stem cuttings from juvenile donor plants was 3.7-8.5 mm. The length of cuttings from both mature and juvenile donor plants was the same (27 cm). Three rooting mediums were used in this experiment, i.e. soil, soil and manure in ratio 1:1, and coco peat.



Figure 1. Stem cuttings of *Sterculia quadrifida* planted in the plastic container then covered by transparent plastic

Experimental procedure

The experiment used a completely randomized design, consisting of nine treatments, and each treatment comprised of 20 stem cuttings as replicates. There were three treatments of the sources of donors and the sizes of diameter (juvenile cutting, small diameter of mature cutting and large diameter of mature cutting); and three treatments of rooting media (soil, soil, and manure (1:1), coco peat). Thus, a total of 180 cutting was observed.

The stem cuttings were soaked in 1% Atonik solution for 30 minutes before planted in the plastic container. Each plastic container represents one treatment, so nine plastic containers were used in this experiment. The plastic container was then covered by transparent plastic to maintain the temperature and humidity of rooting media, as shown in Figure 1. Subsequently, all stem cutting in the plastic containers were kept in the propagation chamber made of wooden stick structure and covered with transparent plastic and paranet for ten weeks. The plastic chamber was used to protect the cuttings from both rainfall and direct sunlight. The stem cuttings were watered every alternate day to avoid desiccation.

Data recording and analysis

The success of stem cuttings was assessed for survival rate, callus formation, and rooting. Data collected in this study included rooting (number of roots, root length and percentage of rooting), callus formation (number of nodes, number of leaves and leaf size), daily temperature, and humidity. The data obtained were tabulated to calculate the survival rate, the percentage of stem rooting and the average temperature and humidity. While the analysis of variance (ANOVA) was carried out using SPSS 16 software package to test for a significant effect of treatment on the number of nodes, the number of leaves, leaf size and root length.

RESULTS AND DISCUSSION

The survival rate of *S. quadrifida* stem cutting is presented in Table 1. Table 1 showed that at the end of observation (10th week), the survival rate of stem cuttings from the juvenile donor and mature donor plant with large diameter (15%-25%) was higher than stem cuttings from mature donor plants with small diameter (0%-5%). The rooting medium had no significant effect on the survival rate of *S. quadrifida* stem cuttings (Table 1).

Table 1. The survival rate of *Sterculia quadrifida* by stem cutting

Rooting medium	Survival rate (%) in 10 th week		
	Juvenile	Mature, large diameter	Mature, small diameter
Soil	25	15	5
Soil + manure (1:1)	20	25	5
Coco peat	20	15	0

The stem cuttings began to grow in the first week, indicated by the bud growing, as showed in Figure 2. Then in the second week, the new leaves started to grow (Figure 3). In the third week, almost all of the stem cuttings were growing but started in the fifth week the survival rates decreased continuously (Figure 4) because the cuttings failed to produce roots (Figure 5). According to literature study, the first differentiation during vegetative

propagation (stem cutting) is buds and leaf growth, which depend on nutrition reserved in the stem, then followed by root development (Swamy et al. 2002). Hence, when the root formation is left behind from the buds and leaf growth, the survival rate will decrease. The results indicated that the source of donor plants and the diameter of stem cutting had significant effects on the survival rate.

Table 2. The effect of donor plant and rooting medium on the number of the root, root length, number of nodes, number of leaves, leaf size and the bud height among the survived stem cuttings (10th-week observation)

Donor plant	Rooting medium	Number of root/cutting	Root length	Number of Nodes	Number of leaves	Leaf size	Bud height
Juvenile	Soil	2	2.1	1.9	3	3.8x6.2	5.4
	Soil: Manure (1:1)	8	2.4	1.8	2.3	4.4x6.8	5.2
	Coco peat	1.7	3.8	2	2	4.8x7.5	6.5
Mature large diameter (15.4-24.4 mm)	Soil	5.5	3.7	3.5	3.5	6.4x8.7	16
	Soil: Manure (1:1)	5.5	2.3	3.7	5	6.4x10.5	13.1
	Coco peat	5.5	3	4	5	5.9x9.3	13.5
Mature small diameter (5.8-13.8 mm)	Soil	2	1.8	2	4	5.3x7.2	6.5
	Soil: Manure (1:1)	5	2.4	3	4	6.1x9.1	13
	Coco peat	0	0	0	0	0	0



Figure 2. The buds were growing in one week after transplanting



Figure 3. The new leaves started to grow in two weeks after transplanting

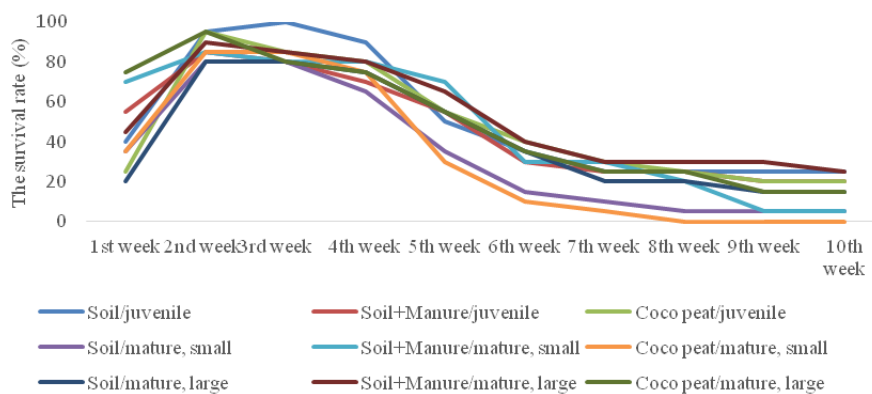


Figure 4. The survival rates of *Sterculia quadrifida* stem cuttings were decreasing from week fifth forward



Figure 5. *Sterculia quadrifida* stem cuttings failed to form roots. The donor plants were taken from mature plants with a small diameter

The stem cuttings from juvenile donor plants effectively improved the survival rate and rooting ability; this result is in line with studies on vegetative propagation of other species such as *Robinia pseudoacacia* and *Grewia optiva* (Swamy et al. 2002), *Dalbergia melanoxylon* (Amri et al. 2010) and *Tectona grandis* (Husen and Pal 2006). Likewise, the cuttings with large diameters derived from mature donor plants also sufficiently increased the survival rate since they had enough reserved food for rooting formation. Similar to research on *Ficus roxburghii* (Rana and Sood 2011) and *Picea abies* (OuYang et al. 2015) that the diameter size increased the survival rate and rooting percentage.

At the end of observation, rooting variables and leaves growth were measured as shown in Table 2. ANOVA revealed that the measured variables of survived cutting were not significantly different among the treatments. The cuttings taken from juvenile donor plants had a better ability to form roots, while the stem cuttings collected from a mature donor with large diameter were better on the above-ground growth variables (shoots and leaves). However, rooting mediums had no consistent effect on the root formation of *S. quadrifida* cutting. Compared to the previous study on stem cutting propagation (Siswadi et al. 2017), the survival rate of stem cuttings in this experiment was increased from up to 6.7% to up to 25%. The average temperature in the plastic tunnel was lower (29°C) than in the previous study (31°C) while the humidity was relatively the same around 85%. This experiment was conducted in the wet season while previously was in the dry season, which may affect the survival rate. The study revealed that time, when the donor plants were collected, plays an essential role in increasing root formation (Swamy et al. 2002).

In conclusion, this experiment showed that the donor plants affected the survival rate of stem cutting of *S. quadrifida*. While the rooting mediums had no significant effect on the survival rate. Stem cutting from juvenile donor plants and mature donor plants with large diameters produced a considerably high percentage of survival rate up to 20%. Stem cuttings from juvenile were sufficient in producing roots, whereas stem cuttings from mature donor plant large-diameter were better in producing buds, leaves, and nodes. Thus, *S. quadrifida* is amenable to vegetative propagation.

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Nutrient and anti-nutrient compositions of cowpea (*Vigna unguiculata*) and mung bean (*Vigna radiata*) seeds grown in humid Southeast Nigeria: A comparison

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Abstract. Enyiukwu DN, Chukwu LA, Bassey IN. 2020. Nutrient and anti-nutrient compositions of southern pea (*Vigna unguiculata*) and mung bean (*Vigna radiata*) seeds grown in humid Southeast Nigeria: A comparison. *Trop Drylands* 3: 41-45. Pulses are important components of African cuisines. Cowpeas (*Vigna unguiculata* (L.) Walp.) are commonly consumed in most parts of Nigeria, but mung bean (*Vigna radiata* (L.) Wilczek) though new in the agro-landscape, is gradually gaining popularity. Its popularity is based on the speculation that it is of higher nutritional content than cowpeas. This work evaluated the nutritional compositions of seeds of both pulse crops grown in humid southeast Nigeria using classical and spectrometric analysis. Results obtained from the study showed that the mean proximate compositions of mung bean (17.70%) did not statistically ($P \geq 0.05$) differ from that recorded for cowpea seeds (17.30%). However, irrespective of the high magnesium content (62.39 mg g^{-1}) of mung bean, the mean mineral nutrients in cowpea (270.69 mg g^{-1}) is grossly higher than (145.58 mg g^{-1}) recorded in mung bean due largely to high presence of potassium ($1.29 \text{ g } 100 \text{ g}^{-1}$) in cowpeas than in the former. Data from this study also showed that higher contents of flavonoids (11.31 mg g^{-1}) make the mean content of phytochemicals in mung bean (4.21 mg g^{-1}) to be slightly higher than but statistically ($P \geq 0.05$) at par with that obtained in cowpea seeds (3.67 mg g^{-1}). Generally, the nutrient contents in mung beans are not substantially higher than that of cowpea and vice versa. It is therefore recommended based on data obtained from this study that both be used to supplement each other in African diets due to their differential contents of flavonoids, magnesium, and potassium for better well-being and health.

Keywords: Cowpea, green gram, mung bean, nutrient composition, southern pea

INTRODUCTION

Southern pea or cowpea (*Vigna unguiculata* (L.) Walp.) and green gram or mung bean (*Vigna radiata* (L.) Wilczek) are members of the same botanical family, i.e., Fabaceae. Cowpea originated from West or Southern Africa, while the later speciated from Indo-Burma; from these centers of origin they spread throughout the tropical world (Sha et al. 2011; Enyiukwu et al. 2018a). Both crops are short-duration crops conspicuously present in many farming systems of the tropics where they function as bio-fertilizers in marginal soils, as forage crops for animal husbandry and catch crops between seasons (Islam et al. 2011; Enyiukwu et al. 2018b).

Cowpea and mung bean may be prepared in diverse food forms, either as vegetables for soups and sauces or pulses (Dakora and Belane 2019; Owade et al. 2019). Pulse-derived flours of these crops, for instance, have also been reported to be used in making noodles, bread, and soups (Abbas and Shah 2007). Medicinal properties relating to anti-hypertensive, anti-diabetic, anticancer, anti-hepatitis, anti-gastritis and body weight regulation have been ascribed to eating some of these legumes (Shasheen et al. 2012; Noel et al. 2019).

Cowpea and mung bean are important sources of energy (calories), proteins, vitamins (A, B-complex, C and K); and minerals such as iron, copper, magnesium, manganese and phosphorus in human diets (Abbas and Shah 2007; Oghbaei and Prakash 2016; Dakora and Belane 2019). Generally, proteins derived from grain legumes are rich in several amino acids, such as lysine and tryptophan; but deficient in sulfur-based amino acids methionine and cysteine – a factor that makes these grains slightly inferior to animal-derived proteins (FAO 2018). In meat-scarce communities of the tropics, blending or supplementing pulse grains with cereals, such as fonios which contain substantial amount of these sulfur-based amino acids, is strongly warranted for balancing diets (FAO 2018; Enyiukwu et al. 2018a, 2020). Also, pulses contain low amounts of saturated fat, sodium, cholesterol but considerable quantities of several non-nutritional compounds (NNCs) including protease inhibitors, lectins, phenolics, phytates, tannins, saponins and alkaloids such as vicine and convicine which are concentrated in their seed coats and cotyledons. Though termed NNCs, these phytochemicals have been associated with contributing to prevention of certain diseases such as cancers, inflammation, hypertension, diabetes, and hyperlipidemia

in humans (Tajoddin et al. 2010; Gulewicz et al. 2014; Jayanthlake et al. 2018). Phytates, for instance, have been reported to demonstrate protection against DNA damage, phenolics demonstrate antioxidant activity and prebiotic activity has been ascribed to galato-oligosaccharides; making these classes of compounds to be touted as nutraceuticals (Campos-Vegas et al. 2010; Dhole and Srinivasalu 2015). Due to their high contents of NNCs, these sources remarked that frequent consumption of these legumes (4 or more times per week) has been significantly and substantially linked with lower risks of coronary heart disease (CHD) or cardiovascular disease (CVD). Nevertheless, several farm households in third-world nations including Pakistan and Uzbekistan have been reported to have little knowledge of the health benefits of consuming these legumes (Rani et al. 2018).

However, some researchers argued that NNCs, especially phytates and polyphenols, could bind or make some kinds of mineral nutrients unavailable to consumers. Oligosaccharides, such as raffinose, starchose and verbasose present at 5-8% on dry matter basis in pulse seeds, cause gastro-intestinal discomforts and flatulence (CO_2 , H_2 , and CH_4) in man and monogastric systems (Mubarak 2005; Ofuya 2006; Sha et al. 2011; Hussain and Burhanddin 2011; Shashen et al. 2012; Akerue and Onwuka 2013). Processing them before eating, particularly sprouting, cooking, roasting, dehulling, de-husking, soaking, and milling (Blessing and Gregory 2010; Tajoddin et al. 2016), could reduce flatulence and other negative effects of these factors (Mubarak et al. 2005; Olanipekun et al. 2013) and improve digestibility and nutrient availability from the grains (Oghbaei and Prakash 2016).

Interest in mung beans as veritable functional food is reported to be growing in China (Shi et al. 2016). In recent

times also in Nigeria, the economic and food value of mung bean is rising being even advanced in some quarters as a superior form of grain legume (Chukwu 2019) with complete protein (Ullah et al. 2014). Therefore as part of efforts by scientists to document nutritional information of locally grown grains, this work analyzed and compared the nutrients and anti-nutrient contents of seeds of cowpea and mung bean grown in the humid Umudike area of Southeast Nigeria.

MATERIALS AND METHODS

Source of seeds and preparation of seed samples for analyses

The experiment was conducted at the Analytical Laboratories of the Federal Institute for Industrial Research Oshodi (FIIRO), Lagos, and National Root Crops Research Institute, (NRCRI) Umudike, Nigeria. The seeds of Cowpea (Var. IAR-48) (Fig. 1A) and mung bean (Var. SML-668) (Fig. 1B) were obtained from the Research and Training (R&T) Unit of the College of Crops and Soil Sciences, Michael Okpara University of Agriculture, Umudike were used for the study. One hundred grams of each seed specimen was weighed out separately with a digital balance. Thereafter, they were separately and repeatedly ground into powder with a hand milling machine (Corona Lavesch 250) (Amadioha and Enyiukwu 2019a, b). The powdered specimens were then stored in air-tight bottles and kept in dark wooden cupboard in the laboratory away from UV light which could degrade some of the phytochemical compounds (Enyiukwu et al. 2018a, b).



Figure 1. Pulse grains observed in this study. (A.) Dried seeds of cowpea; (B.) Dried seeds of mung bean; Photo: by DN Enyiukwu

Biochemical analyses of samples

Standard methods based on the protocols of A.O.A.C (2000) were used in the determination of the proximate compositions (moisture, protein, fat, crude fiber, ash, and carbohydrate contents) of the test specimens. The samples were first scanned with digital moisture analyzer (Model: MS-70, A & D Co. Ltd., UK) fitted with light-emitting diode (LED) for their optimum temperature (140°C), and their percentage moisture contents were determined and displayed automatically on the LED. One gram (1 g) of each ground seed sample was then charred to complete decarbonization on a hot plate, and then put in the furnace (Model 186A, Fisher Scientific Co.) set at 560°C to obtain their ashes (Amadioha and Enyiukwu 2019a). The ashes were then separately dissolved in 1N nitric acid and analyzed for calcium, zinc, magnesium, phosphorus, and iron-based on their individual absorbances from the Atomic Absorption Spectrometer (Model: AA 7000, Shimadzu, Japan). Standard and calibration curves generated from the absorbances of the respective samples and standards were then used to determine the respective amounts of the test elements in the samples (Kayode et al. 2008; Enyiukwu et al. 2018a, b). The test for alkaloids, flavonoids, tannins, saponins, and phenols contents of the seed specimens of the legumes were also determined; by separately subjecting 10 g of each test powder to classical and spectrometric analyses using the procedure described by Enyiukwu and Awurum (2013).

Statistical analysis

This experiment was designed as Completely Randomized Design (CRD) with three replicates. Means of the data collected in the study were analyzed by analysis of variance (ANOVA). The statistical package employed was Genstat (Genstat Release Windows/PC Vista, version 12.10). Means were separated and compared using Fisher's LSD at probability of 0.05.

RESULTS AND DISCUSSION

Results of the proximate nutrient contents of the test pulses are presented in Table 1. It shows that moisture contents of the test pulse seeds ranged between 11.45-12.06%. The data also revealed that protein and carbohydrate were the most abundant nutrients in the pulse seeds, followed by crude fiber while ash and fat were the least. Both seeds of the pulse crops contain ash and fat at statistically ($P \geq 0.05$) par levels. In terms of protein, carbohydrate, and fat, cowpea recording 25.68, 57.00 and 1.70% was statistically ($P \geq 0.05$) equal in its contents of these nutrients with mung bean where 26.25, 55.30, and 2.13% were obtained for these parameters respectively. Presence of crude fiber in cowpea, however, was statistically ($P \geq 0.05$) inferior compared to value obtained in seeds of mung bean (Table 1). Generally, both seeds are low in contents of fats (1.70-2.13%) but high in protein (25.68-26.25%) and low glycemic index carbohydrates (55.98-57.00%).

The results of the mineral profiling of the test pulse seeds are presented in Table 2. It indicated that K identified at 1.292 mg 100 g⁻¹, P (498.06 mg g⁻¹) and Ca (93.10 mg g⁻¹) were the most abundant elemental nutrients in the pulse seeds, and these were significantly ($P \leq 0.05$) superior to the corresponding values of the same parameters in mung bean (Table 2). With the exception of Fe where 11.00 mg g⁻¹ recorded for cowpea was statistically ($P \geq 0.05$) equal with 10.06 mg g⁻¹ obtained for mung bean; values obtained for Na and Mg were significantly higher in mung bean than the corresponding values of the same elements in cowpea (Table 2). In general, the mean elemental nutrients (145.58 mg g⁻¹) recorded in mung bean is statistically ($P \geq 0.05$) inferior to (270.69 mg g⁻¹) obtained in cowpea.

Outcomes of the anti-nutrients evaluation of the test pulses are presented in Table 3. It showed that high levels of flavonoids (11.31 mg g⁻¹) and saponins (7.71 mg g⁻¹) were recorded in seeds of mung bean than 7.96 mg g⁻¹ and 6.09 mg g⁻¹ obtained for the respective parameters in cowpea. Also, 0.76 mg g⁻¹ of tannins recorded in cowpea was slightly but statistically ($P \leq 0.05$) greater than 0.23 mg g⁻¹ obtained in mung bean. However, values obtained for alkaloids, phytates, and oxalates were statistically ($P \geq 0.05$) at par for the test pulse seeds. Generally, the mean NNCs in mung bean (4.31 mg g⁻¹) is slightly ($P \leq 0.05$) higher than the mean value (3.63 mg g⁻¹) in cowpea.

Table 1. Proximate composition of cowpea and mung bean grown in Umudike, southeast Nigeria

Nutrients (%)	Mung bean	Cowpea
Moisture	11.45	12.06
Protein	26.25	25.68
Carbohydrate	55.98	57.00
Fat	2.03	1.70
Crude fiber	6.75	3.94
Ash	3.65	3.41
Mean	17.70	17.30
LSD (0.05)	0.43	0.45

Note: *Data are means of triplicate determinations from 2 separate experiments

Table 2. Mineral composition of seeds of cowpea and mung bean grown in Umudike, southeast Nigeria

Minerals contents (mg g ⁻¹)	Mung bean	Cowpea
Calcium	86.71	93.10
Phosphorus	378.01	498.06
Zinc	1.05	0.17
Sodium	1.65	0.13
Magnesium	62.37	0.09
Iron	10.06	11.00
Potassium**	479.23	1292.25
Mean	145.58	270.69
LSD (0.05)	0.78	1.22

Note: *Data are means of triplicate determinations from 2 separate experiments; **values in mg 100 g⁻¹

Table 3. Phytochemical and anti-nutrient factors in seeds of cowpea and mung bean grown in Umudike, southeast Nigeria

Anti-nutrients/ phytochemicals (mg g ⁻¹)	Mung bean	Cowpea
Alkaloids	6.45	6.87
Flavonoids	11.31	7.96
Tannins	0.23	0.86
Saponins	7.12	6.19
Phytate	0.02	0.03
Oxalate	0.11	0.09
Mean	4.21	3.67
LSD (0.05)	0.11	0.08

Note: *Data are means of triplicate determinations from 2 separate experiments

Discussion

Mineral, proximate and other compositions of legumes are reported to be influenced by genetic especially species or varietal factors and environmental parameters (Abbas et al. 2007; Dhole et al. 2015). Nutrients profiling in this study revealed that the test pulse seeds contain substantial amounts of proximate compounds, a variety of minerals, and anti-nutrient factors; thus confirming the reports of Otitaju et al. (2015), Ayogu et al. (2016), and Dakora and Belane (2019) where all these factors were identified in seeds of locally grown legumes in Nigeria, and other parts of Africa. The high contents of protein and carbohydrates in both seeds of the pulse crops agree with observations by Otitaju et al. (2015) and FAO (2018) where these nutrients were reported in high amounts also. Finding in this study also showed that levels of protein, carbohydrates, fat, and ash did not significantly ($P \geq 0.05$) differ in both pulse seeds; a trend which also conformed with FAO (2018) where these parameters did not differ ($P \geq 0.05$) with each other in the pulse seeds. However, substantially ($P \leq 0.05$) higher presence of crude fiber in mung bean compared to cowpea in this study (Table 1) did not agree with the trend where it was only marginally higher (4.8% and 4.2% respectively) as noted in FAO (2018). Carbohydrate delivers energy to consumers (Abbas and Shah 2007). They are made up of sugars and polymers such as oligo-saccharides. Galacto-oligosaccharides for example play significant roles in nutrition as prebiotics and preventers of some forms of cancers (Campos-Vegas et al. 2010; Enyiukwu et al. 2018a,b). In general, oligosaccharides are present in legumes in the range of 5-8% (Mubarak 2005); some including raffinose, stachyose and verbanose are difficult to break down by human enzymes complex resulting in flatulence. Lower values of these flatulence causing oligosaccharides are reported to occur in mung beans than cowpea (Shasheen et al. 2012), and this may have accounted for the lower value of carbohydrate (55.98%) obtained in this study; and thus might be one reason for advancing mung bean as of higher nutritional quality than cowpea.

Both pulse seeds analyzed in this study are rich sources of valuable mineral elements (Ca, P, K, Mg, Zn, and Fe). However, cowpea seeds are exceptionally rich in potassium (1.29 g 100 g⁻¹) compared to 479.23 mg g⁻¹ in mung bean seeds; whereas mung bean seeds on the other hand are

significantly superior in content of magnesium (62.37 mg g⁻¹) (Table 2). This agrees with the reports of Alayande et al. (2012) that K occurring at 741 mg 100 g⁻¹ was the highest occurring mineral in white-coated seeds of cowpea. It is also supported by Olayiwole et al. (2012) who noted potassium content of up to 1.38 g 100 g⁻¹ in flours derived from some seeds of cowpea used for improving tuber-based recipes in Nigeria.

Anti-nutrient factors, such as alkaloids, flavonoids, tannins, saponins, oxalates, phenolic compounds, and phytates, are associated with seeds of legumes or pulses (Okwu and Orji 2007; Campos-Vegas et al. 2010; Towo et al. 2013; Ayogu et al. 2016) usually in amounts which correlates with the seed color (Dahiya et al. 2015). Though NNCs are commonly regarded as reducing the nutrient quality of pulses by affecting protein digestibility and mineral bio-availability (Towo et al. 2003); some of these compounds are held up in recent research findings as potent antioxidants, prebiotics and nutraceuticals (Campos-Vegas et al. 2010). With the exception of flavonoids which were significantly higher in mung beans, the mean anti-nutrient factors were not substantially ($P \geq 0.05$) different from each other in both pulse seeds under evaluation. Flavonoids give brilliant colors to flowers and seeds; and have been reported to actively participate in maintaining seeds viability and priming body defense systems against pathogenic invasion in humans (Enyiukwu and Awurum 2013; Tajoddin et al. 2010). Perhaps the high flavonoid content of mung bean may explain in part the reason why mung bean is touted as a superior legume over cowpea.

Overall, findings from the nutritional profiling conducted in this study did not seem to suggest any profound superiority of mung bean over cowpea as held in some quarters and vice versa. With the exception of potassium and flavonoids, both seeds contain significant quantities of nutrients (proximate, mineral, and phytochemical compounds) which did not differ substantially ($P \geq 0.05$) from each other. Hence, based on the above findings, it is recommended that consumption of both seeds should be supplemented with the other in our local diets so as to boost cell electrical conductivity, improve cardiac health, and general body immunity.

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Use of probiotics in fish feed and clams (*Pilsbryconcha exilis*) as biofilter components of aquaponics system in archipelagic dryland

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Abstract. Santoso P, Sunadji. 2020. Use of probiotics in fish feed and clams (*Pilsbryconcha exilis*) as biofilter components of aquaponics system in archipelagic dryland. *Trop Drylands* 3: 46-50. Aquaponics is a combination system between aquaculture and hydroponic. It is water-use efficient and suitable to be developed in the archipelagic dryland. This study aimed to determine the effect of adding probiotics in fish feed and the use of clam (*Pilsbryconcha exilis*) as additional components in the aquaponic system on feed conversion ratio, productivity of catfish (*Clarias gariepinus*) and aquatic spinach (*Ipomoea aquatica*), and removal efficiency of organic matter suspended in the aquaculture water. This study was an experimental study using a factorial treatment laid out in a completely randomized design with three replications. The treatments applied were administration of probiotics with concentrations of 2 mL kg⁻¹, 4 mL kg⁻¹, and 6 mL kg⁻¹ in feed to improve feed efficiency in catfish, thus minimizing metabolite discharges, and the use of clam in biofilter systems with a density of 50 clams m⁻², 100 clams m⁻², and 200 clams m⁻² to improve the efficiency of absorption of cultivation effluents in the form of suspended particles. The results of ANOVA showed that the treatment of adding probiotics in the fish feed had a significant effect ($P < 0.05$) on improving feed conversion ratio, increasing the production of catfish, and the removal efficiency, but had no significant effect ($P > 0.05$) on aquatic spinach biomass production. Whereas the use of clams as biofilter significantly ($P < 0.05$) improved feed conversion ratio, increased the production of catfish and aquatic spinach, also efficiently removed organic matter in the aquaponic system. The interaction of these two factors had a significant effect ($P < 0.05$) on feed conversion ratio and aquatic spinach production, however, it had no significant effect ($P > 0.05$) on the production of catfish and the organic matter removal efficiency. Post hoc LSD test showed that the best interaction effect was the treatment of probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻² because it generated a combination of the lowest feed conversion ratio (1.07) and the highest of aquatic spinach production (5.28 kg m⁻²).

Keywords: aquaponic, biofilter, clam, probiotic, production

INTRODUCTION

East Nusa Tenggara is an archipelagic province in Indonesia dominated by dryland areas. This province has a semi-arid climatic type with long dry period and low rainfall. As a consequence, food production through farming, fisheries and livestock rearing needs to be developed to minimize water use. In doing so, it requires appropriate technologies to overcome limited arable land and water limitations, especially in the dry season.

One of the appropriate technologies for food production in water-limited areas with scarce arable land is aquaponic. The aquaponic is an agricultural production system that combines aquaculture and hydroponic simultaneously using same land and water as economically as possible. The ability of aquaponics to efficiently utilize land and water is through the use of waste from fish farming activities by the existing plants (Rakocy et al. 2006). Waste derived from fish farming activities is used as input of organic nutrients to support the growth of plants, such as aquatic spinach. Subsequently, the absorption of fish metabolic waste by aquatic spinach, water quality is improved to be used in fish farming. This mutual benefit makes aquaponics suitable to be developed in areas where

freshwater sources and land are limited such as in densely populated urban settlements.

The selection of commodities plays an important role in the yield of agricultural production. In the context of aquaponics, the types of fish suitable to be maintained are fish that are able to live in sub-optimal water conditions, while the plants usually used are those that have economic value such as aquatic spinach, lettuce, and spinach (Rakocy, et al. 2006). Besides considering both the fish and plant, aquaponic also needs to take into account the optimal conditions for other biotic components of the aquaponic system, namely decomposing bacteria contained in biofilter units. There are two groups of decomposing bacteria in the aquaponic, namely *Nitrosomonas* and *Nitrobacter*. The presence of these bacteria is determined by the charge of solid or dissolved organic matter in water. If excessive organic matter is loaded, bacteria will need more time to break it down. As a result, substances containing ammonia can accumulate in water and endanger the life of fish before it is broken down by bacteria (Losordo et al. 1998).

To minimize organic material in the water, these materials need to be removed periodically from the system. Solid organic matter can be removed from the system mechanically by screening and sedimentation (Losordo et al. 1998). Organic matter in the form of suspended particles

can also be removed by suspension-eating organisms such as bivalves. Therefore, there is an opportunity to add these organisms to the biofilter component. The combination of biofilters in the form of plants and bivalves is expected to increase the filtration capacity of aquaponic system.

With the above considerations, this study explored the combination of increasing feed efficiency through the addition of probiotics with the addition of biofilter components in the form of bivalves/clams. The results of this study are expected to be useful for the development of an aquaponic system that is more effective in conserving water to support the development of freshwater aquaculture, especially catfish in the archipelagic dryland regions.

MATERIALS AND METHODS

Study area

The research was conducted at the Fisheries Field Laboratory, Center of Excellent of Archipelagic Dryland, University of Nusa Cendana. Water quality analysis was carried out at the Laboratory of Fisheries, Faculty of Marine Science and Fisheries, University of Nusa Cendana.

Procedures

This study was laid out in a completely randomized design with a factorial treatment, and three replications. The treatments applied were the combination of addition of probiotics in fish feed with concentrations of 2 mL kg⁻¹, 4 mL kg⁻¹ and 6 mL kg⁻¹, and the use of clam (*Pilsbryconcha exilis*) as biofilter with a density of 50 clams m⁻², 100 clams m⁻², and 200 clams m⁻². The probiotic treatment was expected to increase feed efficiency in catfish (*Clarias gariepinus*) so as to minimize metabolite discharges, whereas the biofilter was used to increase the efficiency of absorption of planting waste in the form of suspended particles.

The aquaponic system consisted of the following components: (i) Fish rearing ponds measuring 3 x 2 x 1 m³. (ii) The place for clam and aquatic spinach (*Ipomoea aquatica*) was made of fiberglass multiplex layered. The size of each aquatic spinach basin was 1 x 0.5 x 0.2 m³. Aquatic spinach rearing tubs were filled with 5-7 mm granular gravel media spread evenly covering the tub surface to a thickness of 5 cm. (iii) Mechanical and biological filters. The mechanical filter used was a swirling sedimentation tank. Mechanical filters treated in this study functioned to separate suspended solids in the water. This filter was connected with biological filters that contain media in the form of bio-ball and net sheet. (iv) Submersible pump. The submersible pump with a capacity of 1,500 L h⁻¹ was used to push water from fish culture tanks to the filter unit. (v) Piping network. Piping used for connecting all system components was made of ½ inch and ¾ inch PVC pipe.

Before use, the entire tub was soaked in water for a month and followed by draining and filling with new water. The density of catfish stocking in all treatment units was 100 fish m⁻². The size of the catfish offspring used had a

length of 5-7 cm and weight of 1.5-2.5 g. Before being placed in the treatment unit, the catfish offspring were acclimatized for a month in a 5-ton concrete tub at the study site. Furthermore, catfish was kept for two months by providing commercial feed, where at the beginning of the study, they were given "starter" fish feed, and after two weeks of age the feed was replaced with "initial" fish feed, and the "final" fish feed was given in the following two weeks and was replaced according to the size of the fish until harvesting. The feeding rate was 5% body weight per day. The weight of the pellet given was corrected every week, according to the growth of the fish. During maintenance, no water replacement was carried out, except for those lost due to evaporation.

After the fish tank was scattered with fish and the water turned greenish due to feeding, the aquatic spinach was ready to be planted. Aquatic spinach seeds were immediately buried in gravel until seeding. Seeds were buried at a distance of 15 cm x 15 cm.

Data analysis

Feed conversion ratio

Feed conversion was calculated according to the formula of Pillay (1990) as follows:

$$FCR = \frac{\text{Total Weight of Feed}}{W_t - W_o}$$

Where:

W_o: total weight of fish at the beginning of the study (kg)

W_t: total weight of fish at the end of the study (kg)

Biomass productivity of catfish and aquatic spinach

The biomass productivity of catfish and aquatic spinach was determined at the end of maintenance. (i) Fish biomass production: Calculated as total catfish biomass per pond area (kg m⁻²). (ii) Aquatic spinach biomass production: Calculated as total aquatic spinach biomass per planting area (kg m⁻²).

Removal efficiency

Removal efficiency/absorption efficiency of solid materials. The efficiency of separating solid material by swirling sedimentation was determined according to the formula of Pfeiffer et al. (2008):

$$RE (\%) = (S_{in} - S_{out}) / S_{in} \times 100$$

Where:

RE : removal efficiency (%):

S_{in} : TSS value of water into filter:

S_{out} : TSS value of water out from filter.

The total suspended solids (TSS) analysis was done using APHA (1995) method. In addition, turbidity, pH, temperature, and ammonia were measured.

Data analysis

Data were analyzed using one-way ANOVA and followed by LSD test. Statistical analysis was carried out using SPSS 24 software.

RESULTS AND DISCUSSION

Feed conversion ratio

The results showed that the feed conversion ratio (FCR) of catfish ranged from 1.02 to 1.39. The treatment means of the administration of probiotics and clams as biofilters are presented in Figure 1.

The obtained feed conversion ratio was then subjected to ANOVA to determine the effect of treatments on the conversion ratio of the catfish feed of the aquaponic system under study. The ANOVA results showed that the addition of probiotics in fish feed and the use of clam in biofilter systems, as well as their interaction, had a significant effect ($P < 0.05$) on the feed conversion ratio. The LSD results showed that the lowest feed conversion ratio occurred in the treatment of probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻², and also the treatment of probiotic 6 mL kg⁻¹ and biofilter 50 clams m⁻².

Biomass productivity of catfish and aquatic spinach

The study results revealed that the productivity of catfish biomass ranged between 11.68-14.28 kg m⁻², while the production of aquatic spinach biomass was between 3.51-5.43 kg m⁻². The average productivity of biomass of catfish and aquatic spinach is presented in Figures 2 and 3.

The ANOVA results showed that the addition of probiotics in fish feed had a significant effect ($P < 0.05$) on the productivity of catfish but had no significant effect ($P > 0.05$) on the productivity of aquatic spinach. Whereas the use of clam in biofilter systems significantly affected ($P < 0.05$) both the productivity of catfish and aquatic spinach. The interaction effect of these two factors was not

significant ($P > 0.05$) on the productivity of catfish but was significant ($P < 0.05$) on aquatic spinach. The results of LSD showed that the highest aquatic spinach productivity occurred in the treatment of application of probiotic 2 mL kg⁻¹ and biofilter 100 clams m⁻², which did not differ significantly with treatment combinations of probiotic 2 mL kg⁻¹ and biofilter 200 clams m⁻², probiotic 4 mL kg⁻¹ and biofilter 200 clams m⁻², and also probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻².

Removal efficiency

The results demonstrated that removal efficiency (RE), absorption efficiency of solid materials, ranged from 41.28 to 52.73%. The mean RE values of the treatments are presented in Figure 4.

The results of ANOVA showed that adding probiotics in fish feed and using clam in biofilter systems had a significantly different effect ($P > 0.05$) on the removal efficiency. However, interaction effect of these two factors was not significant ($P > 0.05$) on the removal efficiency.

The results of measurements and analysis of other water quality parameters included turbidity (4.12-6.72 NTU), pH (7.11-8.05), water temperature (25.83-28.07 °C), and ammonia concentration (0.01-0.09 ppm). Water quality parameter values are supporting data in the discussion of the results of this study. These parameters relate to the cycle of organic matter, especially nitrogen in the culture media, so it is important to understand the phenomena that occur and determine the development of the aquaponics system.

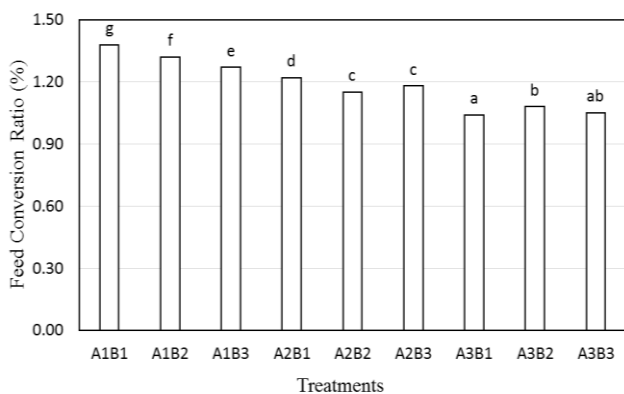


Figure 1. Feed conversion ratio in catfish. Treatment's code: A₁B₁ (probiotic 2 mL kg⁻¹ and biofilter 50 clams m⁻²); A₁B₂ (probiotic 2 mL kg⁻¹ and biofilter 100 clams m⁻²); A₁B₃ (probiotic 2 mL kg⁻¹ and biofilter 200 clams m⁻²); A₂B₁ (probiotic 4 mL kg⁻¹ and biofilter 50 clams m⁻²); A₂B₂ (probiotic 4 mL kg⁻¹ and biofilter 100 clams m⁻²); A₂B₃ (probiotic 4 mL kg⁻¹ and biofilter 200 clams m⁻²); A₃B₁ (probiotic 6 mL kg⁻¹ and biofilter 50 clams m⁻²); A₃B₂ (probiotic 6 mL kg⁻¹ and biofilter 100 clams m⁻²); A₃B₃ (probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻²).

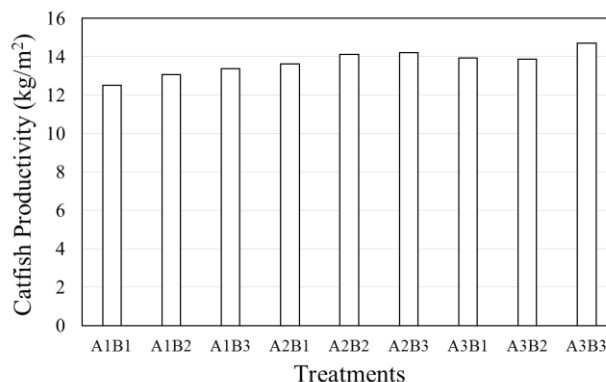


Figure 2. Biomass productivity of catfish. Treatment's code: A₁B₁ (probiotic 2 mL kg⁻¹ and biofilter 50 clams m⁻²); A₁B₂ (probiotic 2 mL kg⁻¹ and biofilter 100 clams m⁻²); A₁B₃ (probiotic 2 mL kg⁻¹ and biofilter 200 clams m⁻²); A₂B₁ (probiotic 4 mL kg⁻¹ and biofilter 50 clams m⁻²); A₂B₂ (probiotic 4 mL kg⁻¹ and biofilter 100 clams m⁻²); A₂B₃ (probiotic 4 mL kg⁻¹ and biofilter 200 clams m⁻²); A₃B₁ (probiotic 6 mL kg⁻¹ and biofilter 50 clams m⁻²); A₃B₂ (probiotic 6 mL kg⁻¹ and biofilter 100 clams m⁻²); A₃B₃ (probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻²).

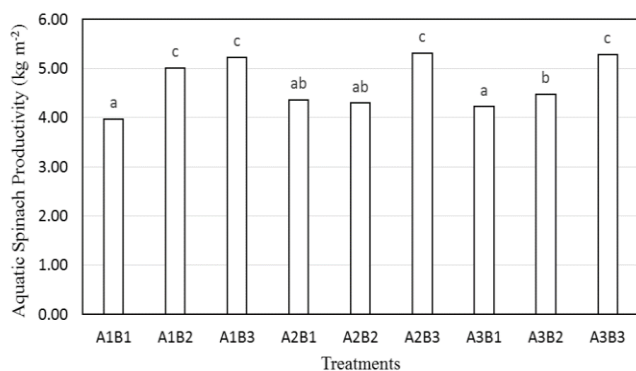


Figure 3. Biomass productivity of aquatic spinach. Treatment's code: A₁B₁ (probiotic 2 mL kg⁻¹ and biofilter 50 clams m⁻²); A₁B₂ (probiotic 2 mL kg⁻¹ and biofilter 100 clams m⁻²); A₁B₃ (probiotic 2 mL kg⁻¹ and biofilter 200 clams m⁻²); A₂B₁ (probiotic 4 mL kg⁻¹ and biofilter 50 clams m⁻²); A₂B₂ (probiotic 4 mL kg⁻¹ and biofilter 100 clams m⁻²); A₂B₃ (probiotic 4 mL kg⁻¹ and biofilter 200 clams m⁻²); A₃B₁ (probiotic 6 mL kg⁻¹ and biofilter 50 clams m⁻²); A₃B₂ (probiotic 6 mL kg⁻¹ and biofilter 100 clams m⁻²); A₃B₃ (probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻²)

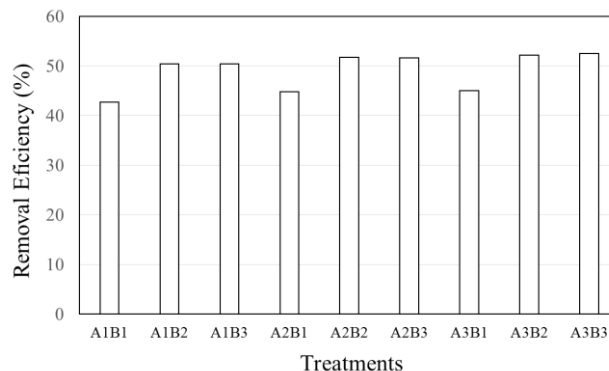


Figure 4. Removal efficiency in aquaculture media water. Treatment's code: A₁B₁ (probiotic 2 mL kg⁻¹ and biofilter 50 clams m⁻²); A₁B₂ (probiotic 2 mL kg⁻¹ and biofilter 100 clams m⁻²); A₁B₃ (probiotic 2 mL kg⁻¹ and biofilter 200 clams m⁻²); A₂B₁ (probiotic 4 mL kg⁻¹ and biofilter 50 clams m⁻²); A₂B₂ (probiotic 4 mL kg⁻¹ and biofilter 100 clams m⁻²); A₂B₃ (probiotic 4 mL kg⁻¹ and biofilter 200 clams m⁻²); A₃B₁ (probiotic 6 mL kg⁻¹ and biofilter 50 clams m⁻²); A₃B₂ (probiotic 6 mL kg⁻¹ and biofilter 100 clams m⁻²); A₃B₃ (probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻²)

Discussion

Adding probiotics in fish feed had a significant effect on improving feed conversion ratio, increasing the production of catfish, and the removal efficiency, but had no significant effect on aquatic spinach biomass production. The application of probiotics in fish feed during one culture cycle has been proven to reduce the value of feed conversion ratio in catfish (Fackri et al. 2016; Fadholi et al. 2016; Lili et al. 2018). Probiotics mixed in fish food facilitated the absorption process of feed nutrients in the digestive tract and fish's stomach, so that the feed provided was more efficient in stimulating the growth of catfish. Probiotic bacteria used in this study were *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Bacillus subtilis*, *Aspergillus oryzae*, *Rhodopseudomonas*, *Actinomyces*, and *Nitrobacter*. Probiotic bacteria such as *Bacillus* can increase feed digestibility by producing protease enzymes in the digestive tract of fish (Wardika et al., 2014). In addition, the presence of probiotic bacteria also plays a role in improving fish health, because the presence of probiotic bacteria can suppress the population of pathogenic bacteria (Cruz et al. 2012).

Some probiotic bacteria, such as *Nitrobacter* that enter the culture media also play a role in the process of overhauling organic material. This bacterium acts as a decomposer that remodels metabolites and remnants of food waste in aquaculture media into the form of nitrates (NO₃⁻) which can be utilized by vegetables in biofilter components (Tyson et al. 2004). Thus, metabolite discharges and food waste can be used effectively for the growth of vegetables in the biofilter, while the activity of the vegetables themselves can increase the concentration of dissolved oxygen in water. Rakocy (2006) suggested that vegetables need nitrates and phosphates for their growth. Potential of probiotics isolated from Sangkuriang catfish to

improve growth performance and feed efficiency was reported by Manoppo (2018).

The use of clam as biofilter had a significant effect on improving feed conversion ratio, increasing the production of catfish and aquatic spinach, also the removal efficiency in the aquaponic system. Nitrifying bacteria changed fish waste as nutrients that can be utilized by plants. Then, this plant will function as a vegetative filter, which will break down these toxic substances into substances that are not harmful to fish, and supply oxygen (O₂) to the water used to cultivate fish. Thus, this is a cycle of mutual benefit (Tyson et al. 2004). In general, aquaponic uses a recirculation system. That is, reusing water that has been used in fish farming with biological and physical filters in the form of plants and the media. The recirculation used contains maintenance and water treatment compartments. Typically, water treatment systems are composed of decantation compartments, filtration compartments, oxygenation compartments and sterilization compartments.

According to Rakocy (2006), aquatic spinach will grow if the concentration of nitrate (NO₃) and phosphorus (PO₄) compounds is sufficient in the water. The nitrate compound in the aquaponic system is the decomposition of fish excretion and leftover food by bacteria in the water. *Nitrosomonas* and *Nitrobacter* bacteria can work well if there is enough oxygen and not too much organic matter. In the system with whirling sedimentation, solid organic matter is easily trapped in the sedimentation tank so it does not enter directly into the biofilter. This makes the bacteria in the biofilter tankless burdened than the system without swirling sedimentation because it only decomposes dissolved organic matter. As a result, bacteria in a system with swirling sedimentation are able to decompose quickly the organic matter which is dissolved into nitrate. Whereas in a system without swirling sedimentation, a high load of

solid organic matter inhibits the work of bacteria in breaking down organic matter into nitrates. As a result, aquatic spinach production is lower in this system.

Ammonia in the waters comes from fish metabolism that is dissolved in water, fish feces, as well as from fish food that is not consumed and settles at the bottom of the aquaculture pond (Pillay 2004). There are a number of factors that can cause ammonia concentrations to increase, including decaying inedible fish, decreasing levels of dissolved oxygen in ponds; the dissolved oxygen in the range of 1 - 5 ppm will cause slow fish growth while dissolved oxygen less than 1 ppm can be toxic to most fish species (Rully 2011).

The LSD post hoc test showed that the best interaction effect was the treatment combination of probiotic 6 mL kg^{-1} and biofilter 200 clams m^{-2} because it generated a combination of the lowest feed conversion ratio and the highest of aquatic spinach production. The aquaponics system reduces ammonia by absorbing wastewater or by using plant roots so that the absorbed ammonia undergoes an oxidation process with the help of oxygen and bacteria, ammonia is converted into nitrates (Widyastuti 2008). In aquaculture activities without changing the water with afresh one, bacteria have an important role in removing ammonia particles through the process of nitrification (Rully 2011). Ammonia (NH_4^+) is non-toxic, but its non-ionized (NH_3) form is very toxic (Kordi and Tancung 2007). The decay process was first formed ammonia (NH_3) as a result of the overhaul of amino acids by various types of aerobic and anaerobic bacteria. Demolition will produce a free CO_2 gas. If the condition of the water is getting worse, so that O_2 in the water runs out, then the process of dismantling the organic material will slowly be taken up by another well-known bacteria, i.e., Nitrosomonas into nitrite compounds. If the waters contain enough cations, the nitric acid formed can immediately be converted to nitrite salts by Nitrobacter bacteria, the nitrite salts are then further converted into nitrate salts. Nitrite salts are important as a mineral assimilated by green plants to rearrange amino acids in the body to form the protoplasm.

Increasing the effectiveness of the aquaponics system will support the quality of water that is maintained in accordance with the needs of cultivated catfish. The saving of water used in this aquaponics system is very suitable to the condition of the archipelagic dryland which has a limited supply of freshwater. So, the development and improvement of the aquaponics system are expected to be an alternative in the development of freshwater fish farming in the archipelagic dryland areas.

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Agricultural innovations and adaptation strategies among upland communities in the state boundary of Kupang District (Indonesia) and Oecusse Enclave (East Timor)

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Abstract. Ngongo Y, Markus JER. 2020. *Agricultural innovations and adaptation strategies among upland communities in the state boundary of Kupang District (Indonesia) and Oecusse Enclave (East Timor)*. *Trop Drylands* 4: 51-57. The policy pledge of 'development started from the periphery' has been implemented as a new paradigm of national development in Indonesia. Agricultural Ministry translated this national policy by implementing several programs and one of them is promoting agricultural innovations to boost agricultural production and rural development in the state boundary. This paper elaborates on agricultural innovations being introduced and adaptations of local people towards harsh climate and to protect natural resources in the state boundary of Kupang District of Republic of Indonesia and Oecusse enclave of Republic Democratic of Timor Leste (East Timor). Data and information are used mainly from the on-going program of Agricultural Innovation Support (AIS). Some insights of similar programs implemented in the three other districts of Timor that share borderlines with East Timor have also been used to enrich the narratives. The study shows that agricultural innovation being introduced has successfully been adopted by co-operators, however, it has limited impacts on the surrounding farmers/villages due to farmers' poor access to the external inputs market, and agricultural extensions. This implies that agricultural innovations should consider farmers' circumstances, particularly harsh climate of the region, and it should be compatible with household calendar both in farming and off-farming particularly in honey harvesting. Traditional beekeeping by Amfoang community demonstrates how local people maintain one of the important sources of their livelihoods and at the same time protect natural resources. Recognizing and supporting customary law on honey harvesting helps protect forests and biodiversity in Amfoang and Timor in general.

Keywords: Agricultural innovations, adaptations, fragile ecosystem, State Boundary, periphery

INTRODUCTION

East Nusa Tenggara (ENT) Province, Indonesia in general has specific biophysical characteristics that differ from the western part of Indonesia. The region is dominated by up-lifted coral reef, infertile soils, low and erratic rainfall (Audley-Charles 1965, 1974), making it a fragile ecosystem (Ormeling 1957). These specific characteristics of ENT Province have been less taken into consideration in national development programs at least up to the 1980s.

Despite improvement in terms of food security and income generation of upland farmers, agricultural development programs and better access/transboundary markets have also contributed to the deterioration of some important or specific upland commodities in semi-arid region of East Nusa Tenggara. For example, Timor apple once dominated local market up to 1970s has been infected by *Marsonina* at the end of 1970s and completely destroyed in the 1980s. So'e oranges, well known as JKS or *jeruk keprok So'e*, is recently deteriorated by pests and diseases (Ngongo 2010), and very recently is blood disease of banana in Sumba Island (Mudita and Benu 2018).

East Nusa Tenggara of Indonesian province shares borderline with Republic Democratic Timor Leste (RDTL,

East Timor) around 268.8 km long, which includes around 15.2 km long borderlines between Amfoang region of Kupang District and Oecusse District - an enclave area of East Timor (BNPP 2015). Amfoang region is considered the most isolated area in Timor Island due to lack of basic infrastructure. However, in line with central government commitment to develop Indonesia from the periphery, Amfoang regions recently receive some programs to unlock the physical isolation of the region.

One strategy to improve food production and welfare of the people in the state boundary region is by introducing appropriate agricultural innovations. Since 2013, Ministry of Agriculture through East Nusa Tenggara-Assistance Institute for Agricultural Technology (ENT-AIAT) has supported farmers in the boundaries of West Timor (Republic of Indonesia) and Republic Democratic Timor Leste (RDTL) through introducing/improving agricultural innovation in order to increase agricultural production and farmers' income. It is implemented through Agricultural Innovation Field Laboratory (AIFL) and similar program continues up to recently with so-called Agricultural Innovation Support (AIS) in the state boundary of RI-RDTL (Ngongo et al. 2018). This paper overview agricultural innovations in remote areas of state boundary of RI-RDTL particularly in Kupang District, and

investigate the responses and adaptability of farmers' to the harsh environment.

MATERIALS AND METHODS

The study was conducted in the state boundary of Kupang District (East Nusa Tenggara Province, Indonesia) and Oecusse (East Timor/RDTL). Three villages namely: Netemnanu, North Netemnanu and South Netemnanu of East Amfoang Sub-district, Kupang District, East Nusa Tenggara Province, Indonesia (Figure 1) were chosen purposively based on the closeness or in the borderline with Oecusse enclave of East Timor where adaptive research for food crops (rice and maize) from the Agricultural Innovation Support (AIS) Program was conducted. Data and information used were part of the AIS Program. Baseline data and information gathered prior to the Program was started at end of 2016. The data and information were updated and enriched during Adaptive Research/Program conducted from 2017 up to recently. The research employed descriptive method for data interpretation

Some insights of similar programs implemented in three other districts (Belu, Malaka, and Timor Tengah Utara) that share borderlines with East Timor were also used to enrich the narratives.

RESULTS AND DISCUSSION

Overview of agricultural innovations of the region

Almost all upland farmers in state boundary of Amfoang in Kupang District are still practicing traditional farming practices. Shifting cultivation with slash and burn agriculture is practiced by majority of upland farmers with less or no external inputs use. Slash and burn or shifting cultivation has been practiced in Timor Island in general since the 13th century which can be traced to when Chinese traders reached Timor with the main motive to buy sandalwood (Wolters 1967). This means that agricultural practices in the remote areas of Timor remain unchanged up to recently.

Although upland farming practice remains unchanged, local people were able to incorporate some new crops brought by outsiders, particularly by Chinese and Indian traders who buy sandalwood (Schafer 1963). Introducing new crops continued during the Dutch colony, particularly horticultural crops were mostly planted in the highland of Mutis and Amfoang regions to supply local markets in Timor (Ngongo 2011). Upland farming practices and extraction of natural resources in the region are dual economies in subsistence crop production and source of cash income (Chopin et al. 2017; Shepherd and Palmer 2015).



Figure 1. The study site in East Amfoang Sub-district (red box), Kupang District, East Nusa Tenggara Province, Indonesia

Introduction of new crops before and during the Colonial era had positive impact on the improvement of food security of local communities (Ataupah 1992). However, the introduction of livestock particularly cattle brought improvement of local people income but it negatively impacted the environment (Boxer 1949; Ormeling 1957). Until recently, Bali cattle almost replaced water buffalo in Timor. The region of Amfoang and Mutis becomes traditional breeding areas for Bali cattle, causing major problems for crops farming and environment (Figure 2). Free-ranging cattle in the region limited farmers to extend their cultivation area for crops and forced farmers to invest more labor and money for fencing to protect crops. Free-ranging cattle in the protected forest make it hard to do vaccination and to control transmitted diseases.

In the semi-arid region of Timor, water is the main constraint in food and fodders. During the long dry season, cattle are heavily dependent on rice straw in abandoned paddy fields after harvest. Rice paddy field become

common grazing area for cattle, limiting the chance to develop dry season crops. One of the main innovations needed for this problem is how to provide fodders of good quality.

Leucaena is the main innovation introduced both to provide good quality fodder and to improve soil quality. This innovation is actually started in Amarasi and then spreads across Timor island and other parts of East Nusa Tenggara Province in general (Piggin and Parera 1984). Nevertheless, Leucaena introduction is less adopted by the farmers in Amfoang region. In contrast to the Amfoang region, farmers in the neighboring area of Timor Tengah Utara (TTU) District have adopted and integrated Leucaena into their farming system. Indeed, most of the TTU landscape recently covered with Leucaena (Figure 3), permitting cattle fattening system works and improving soil quality that shortens fallow periods in shifting cultivation practice.



Figure 2. Cattle free grazing in the highland and in the lowland of Amfoang region. A. Free grazing cattle in the highland protected area, B. Free grazing cattle at the abandoned rice field after harvest



Figure 3. Innovation through introduction of Leucaena in TTU. A. Landscape covered with Leucaena in TTU District, B. Leucaena in rocky land and being cut for land preparation of upland farming in TTU District

Key informants in East Amfoang informed that the main reason why Leucaena and related fodders innovations do not work in Amfoang region is due to people feeling that there is enough open access area, including in government-protected areas that can be used for free grazing. They also feel that they have enough fertile soils in the coastal areas for crop farming and then become grazing areas during dry season.

Based on the above account, very limited agricultural innovation is being introduced and adopted by farmers in the Amfoang region. Adoption constraints are more on socio-cultural aspects rather than technical aspects. Therefore, institutional and social engineering should go parallel with the technical aspect of bringing improvement in Amfoang.

Overview of induced agricultural innovation in the state boundary

Of the four districts in West Timor that share borderline with East Timor, Kupang District is the last district where agricultural programs for state boundaries are implemented. Belu District is the district that gets first benefit of the program. Related programs to improve food crops productivity started in Belu in 2013, while in Kupang District started in 2017.

Under the AARD&D-Ministry of Agriculture has launched the Field Laboratory of Agricultural Innovation (FLAI) program in 2013/2014. The FLAI program was first implemented in Belu District as part of the AARD&D's commitment to developing state boundary through accelerating agricultural innovation adoption rate. The main goal of FLAI is to increase the roles of agricultural sector in improving people's welfare in state boundaries through agricultural innovation.

The FLAI program was designed to improve productivity of six main commodities, i.e.: rice, maize, mungbean, horticulture, cattle and forage. All activities have been carried out in the concept of CLS and zero waste, and focused on Raihat Sub-district, Belu District. Adaptive research for those commodities was carried out in the flat land along Malibaka river bank. Innovation being implemented to improve farming practices has significant impact to increase productivity for all commodities. Innovation related to lowland rice farming has profound impact to improve rice production and it has triggered the increase in rice planting area and increase in adoption rate of rice planting innovations.

Besides continuing improving agricultural innovations in specific farming circumstances of Indonesia-East Timor, FLAI program in 2016 was focused to improve food crops (rice, maize, and mungbean) production and to improve seed production. By the program, rice seed producers in Raihat were also able to produce seed that was supplied to the neighboring districts of Malaka and TTU and even to neighboring farmers in Maliana District of RDTL (BPTP-NTT 2016).

Agricultural innovation was introduced in state boundary of Kupang District based on the baseline survey conducted at the end of 2017. It was revealed from the baseline survey that productivity for all commodities is

considered low and almost no external input or new innovations are implemented in the existing farming systems. In these circumstances, AIAT, in collaboration with farmers and extensionists, agreed to conduct adaptive research for staple crops, first: rice and maize, and then will introduce other innovations related to livestock and income generations.

Adaptive research for maize was conducted in South Netemnanu, while rice was conducted in North Netemnanau village of East Amfoang Sub-district in 2017/2018 rainy season. First-year implementation of the program was a challenge due to limitations in human resources (farmers) and extension workers who have passion for staying in the field due to poor infrastructure. Nevertheless, those farmers who were involved in adaptive research (co-operator farmers) showed excellent performance. Implementation of recommended innovations had significant impact on the improvement of productivity and production of both commodities. Productivity of three high yield varieties (HYVs) introduced namely Inpari 9, Inpari 6, and Ciherang increased double (6.6-7.23 ton/ha) compared with local variety (3.26 ton/ha). The productivity of HYVs is consistent in the last three planting seasons. Similar to rice, there was steady increase of introduced maize (Lamuru) productivity of 4-5 ton/ha compared with the existing local variety of maize (0.8-1.2 ton/ha) (BPTP-NTT 2018).

The above account revealed that farmers in semi-arid areas, at least co-operator farmers in the state boundary, are willing to adopt new innovation if it suits their household circumstances. Marginal farmers in semi-arid areas tend to be vigilant and try to minimize risk for any introduced innovation.

Food security and protection of natural resources: Honeybee case

Food security is still the main priority for households and small farmers in general in state boundaries; however, Timorese dominated by Meto tribe in state boundary of Kupang Districts always feels secure in food production. The term of secure or enough for local people does not mean that food production is enough for households. Rather, they try to manage food production and combine them with access to forest products to meet household' food security.

There are several ways for people in state boundary to ensure household' food security. The most common strategy is by planting many possible food crops in a parcel of land or in several parcels of land at farm level. McCord et al. (2015) stated that: "Crop diversification is one strategy that smallholder farmers may employ to reduce their vulnerability in the face of global environmental change." Farming in semi-arid areas visage high risk of failure mainly due to water limitation. Therefore, planting many possible crops ensures that at least one or two crops will be harvested and can minimize vulnerability.

The upland farming practice fully depends on rainfall and therefore it could be practiced during rainy season (November/December-March). Like other places in upland Timor, main food crops in upland are maize, pumpkin, and

beans, however, the composition of the crops varies based on the zone agro-ecosystem and farmers’ experiences on their land. Upland farmers in hillsides of Mutis, for example, plant more roots crops like sweet potatoes and vegetables. No maize is planted in the highland during rainy season due to cool weather and strong wind during monsoon season. Maize planted on limited area in the highland is only during the dry season.

To meet their staple food (maize) needs, people in the highland do have a parcel of land at lower altitude that suits maize. They keep planting root crops and vegetables in the highland both in rainy and dry seasons mainly for cash income (Figure 4). Every household has different sizes of land, species of plants, and business scale.

For Timorese in the state boundary of Amfoang-Kupang District, natural resources, particularly forests, are important parts of food security. Food deficit from upland farming can be compensated from non-timber forest products like *Dioscorea* sp., and most importantly honey. For local people, protecting forests has positive correlation or direct impact on maintaining the traditional honeybee management. Dominant respondent farmers in the state boundary of Kupang District revealed that main aim of honeybees management is to obtain cash income. Other important motives are conserving norms and local traditions, protecting biodiversity, and ecology functions (Table 1).

Tobe (or elders) who are responsible for the management of honey will make an announcement to the community and set calendar from “calling the honeybee,” harvesting up to let “the honeybee return to its first kingdom place.” A ritual to call the honeybee to come in the community’s forest concession is performed in the arranged stone altar.

According to the respondents in Netemnanau village, best honey quality occurs during *Eucalyptus* sp flowering,

and therefore people are strictly prohibited from cut-off even single branch of *Eucalyptus* during this trees flowering. People are also strictly prohibited to cut some trees where honeybees normally hang. Those who are violating traditional rules will be punished by paying animals (normally pigs) and rice. Blood from the slaughtering of animals is considered atonement with the “wounded nature” or to calm down the “angry nature” and then reconcile with nature.

Besides protecting *Eucalyptus* sp. as main source of nectar, elders in Netemnanu village informed that they also strictly protect the trees where the honeycomb normally hangs. They noticed five important trees as *Fanik*, *Neke*, *Nisa/Nitas*, *Niku*, and *Bonak*. Elder Oematan figured out one of the *Fanik* trees (Figure 5) that he predicted it ages around 250-300 years old based on his grandfather’s story.

Besides protecting the forest, local people also protect the honeybees by letting the honeybees reproduce twice before their honey harvest. This strict role is still implemented until recently. They started to harvest the honey when elder oversaw that the honey was mature enough to be harvested. He stated that they normally harvest first of the mature honey in the lowland and then followed with the midland and the last harvested in the highland.

Table 1. Respondents motives in traditional honeybee management (n=45).

Aspect/s	%
Main source of cash income	93
Ecology functions	53
Protecting biodiversity	79
Conserving norms and tradition of honeybee keeping	89



A



B

Figure 4. Various crops planted in the highland. A. Limited maize and various vegetables planted during the dry season in the highland, B. Various crops in a parcel of land during dry season in highland.



Figure 5. Elders Oematan explains how they protect the Fanik tree (ages 250-300 yo) where the honeybees normally hang every year.

Honey is harvested together by a group of people that have a traditional concession forest area. Those involved in honey harvest should follow traditional procedures and, most importantly, have "clean heart." They make a camp in the forest and stay there to complete the harvest for around two weeks. They are harvesting the honey during the nighttime (07.00 pm-04.00 am) while during the day, they are resting and preparing logistics. Harvested honey will be

shared equally with all members of the group.

Elders Oematan in Netemnanau village informed that they do harvest honey twice a year: April-May and September-October. The best quality of honey is harvested during the first harvest season (April/May) where Eucalyptus flowering; while the second harvest occurs during the dry season where limited trees flower. For Elders Oematan, selling honey is main source of cash

income and it helps to compensate for food shortage or crop failure. Crops failure may occur, but no total harvest failure of honey in his forest concession.

Collecting honey in Amfoang and Timor, in general, is considered one of the ancient activities of the local people. Collecting honey has broad impacts not only on the household income but also on the community's environment and social relations (Lyon and Parkins 2013). It is predicted that around 40% of the household involved directly in honey harvesting. According to the elders in Tuamnanu village, they have strict roles in managing the traditional honeybee harvesting business. They are proud that they are able to keep their traditions particularly for hanging honeybees; however, they are quite permissive for cave/underground honeybees.

The position of elders is inherited from his descendants and it cannot be transferred to other members of community. Every elder has its community concession land for generation that can encompass at least one village. The altar where the elder and community members perform ritual for honey bee management in Netemnanu village is part of government forest area; however, local people can freely take the benefit of the forest and at the same time protect the forest.

In conclusion, traditional upland farming system is still practiced in the state boundary of RI-RDTL RIRDRTL since fewer agricultural innovations were introduced in the region. The remoteness or isolation of the region limits the farmers to access agricultural innovations and markets. Nevertheless, recent new paradigm in looking at state boundaries by central government as "front porch" has promoted improvement in many aspects of rural development in the region. Lessons from the AIS program in state boundary showed that there has been increase in maize and rice productivity for co-operators and surrounding farmers who implement recommended innovation. Farmers are willing to adopt any agricultural innovations that suit household circumstances and their environments. Farmers are able to harmonize farming activities and protect natural resources, particularly in relation to honeybee management. Dry-forest dominated by Eucalyptus in Amfoang-Mutis has played important roles in semi-arid ecosystems. It is important to ensure that any government programs to open the isolation of the region by improving infrastructure in the state boundary will maintain balance between the will to increase agricultural production and protection of natural resources.

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The effect of various dosages of fishbone flour and tofu slurry on chemical characteristic of alfisol and yield of leaf cabbage (*Brassica oleracea* var. *acephala*)

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Abstract. Soetedjo INP, Jansari EA, Ndiwa ASS. 2020. *The effect of various dosages of fishbone flour and tofu slurry on chemical characteristics of alfisol and yield of leaf cabbage (Brassica oleracea var. acephala). Trop Drylands 4: 58-61.* Alfisol is a type of soil that has been going through intensive weathering and development, resulting in leaching of nutrients such as N, P, and K. These conditions might be improved by application of organic matters such as various kinds of waste. Fishbone flour and tofu slurry are potential organic waste that might be applied to improve chemical characteristics of alfisol. This research aimed to determine a proper dosage waste of fishbone and tofu slurry in improving chemical characteristics of alfisol and yield of leaf cabbage (kalian or kale) (*Brassica oleracea* var. *acephala*). Factorial experiment was conducted in a Randomized Complete Block Design (RCBD) with 3 replications. The research treatments were two factors, i.e., kinds of organic waste (fishbone flour and tofu slurry) and various dosage applications (300 g polybag⁻¹, 400 g polybag⁻¹, 500 g polybag⁻¹). Observed data were subjected to Analysis of Variance (ANOVA) followed by a Duncan Multiple Range Test (DMRT) at 5% level. Observed variables included soil nutrient content (total nitrogen, available phosphorus, exchangeable potassium) and yield of leaf cabbage. Result of the research showed that there was no significant effect of either interaction of the two factors or the single factor treatment of organic waste on all observed data. However, dosage of organic waste application significantly affected nutrients content of alfisol that 500 g polybag⁻¹ produced a higher content of N, P, and K compared to other treatments. Similarly, application 500 g polybag⁻¹ of organic waste resulted in a higher fresh weight of leaf cabbage of 218.83 g polybag⁻¹.

Keywords: Alfisol, fishbone flour, organic waste, tofu slurry, yield, leaf cabbage

INTRODUCTION

Generally, alfisol and vertisol are two dominant soil types in dryland regions such as in East Nusa Tenggara. Alfisol has low content of nutrients such as N, P, K, C organic, Mo, Mg, and also Al, Fe, and Mn poisonings. Moreover, alfisol has medium to high bulk density, low to medium soil permeability, low water soil resistance, high sensitivity of erosion, and low soil microbe content (Harjowigeno 2015; Serangmo 2015). These conditions cause a low capability of the soil in supporting growth and yield of crops. Numerous ways have been practiced to improve capabilities of alfisol such as applying inorganic fertilizers. However, some studies showed that those practices increased soil compaction, and increased soil microbiology formation (Muyassir et al. 2012; Ramli et al. 2016). These conditions might be improved by application of organic fertilizer. Organic fertilizer might be able to improve physical, chemical, and biological characteristics of soil (Debosz et al. 2001; Abu-Zahra and Tahboub 2008).

One of the important sources of organic matter is household waste such as fishbone flour and tofu slurry (Anonymous 2010; Anas 2017). There are some parts of fish that are commonly unusable such as fishbone, fish head, fishtail, etc. Therefore, they might be a promising way to utilize fish waste such as fishbone as source of

organic fertilizer to minimize environmental impact on coastal areas while at the same time might improve soil physical, chemical, and biological properties. This is mainly because fishbone contains nitrogen, phosphorus, potassium, calcium, and magnesium (Asprilliani 2010).

Utilization of fishbone as organic fertilizer depends strongly on the fish fat content. A higher fat content might affect decomposing processes. FAO has set a standard criterion for fish fertilizer, i.e., it should contain at least nitrogen of 12%, phosphorus of 8%, and potassium of 6%. Moreover, fishbone flour also has a high calcium content of 2.42-2.53%, water content of 2.55- 3.76%, protein content of 16.60-17.51%, fat content of 3.51-6.26%, and ash content of 65.61-67.94%, phosphorus content of 11.34-17%, nitrogen content of 5.0-31.25% and potassium of (3.70-7.0) (Nabil 2015). Therefore, fishbone might be utilized as a potential organic fertilizer.

Tofu slurry is a solid waste produced by tofu industry which becomes an environmental problem if the waste is not managed properly. Meanwhile, some studies reported that tofu slurry has water content of 2.69%, crude protein by 27.09%, crude fiber by 22.85%, fat by 7.37%, ash by 35.02%, calcium by 0.5%, phosphorus by 0.2%, some nitrogen and C organic (Ariliani 2010; Amtiran 2017). Therefore, tofu slurry might potentially be utilized as organic fertilizer similar to fishbone.

Utilization of both wastes as organic fertilizers depends strongly on how much dosage should be applied to improve the chemical properties of alfisol. This is mainly because various types of soil have different physical and chemical characteristics as reported by Tangketasik et al. (2012) and Hardjowigeno (2015). Some types of soil have high contents of clay with low content of some nutrients, and others have a low content of clay and moderate content of some nutrients. Excessive fertilizer dosage might result in unbalanced composition of some nutrients on the soil, which might cause a decrease in exchangeability of cation and anion, unavailable for supporting growth and yield of plant. Therefore, this research aimed to determine a proper dosage waste of fishbone and tofu slurry in improving chemical characteristics of alfisol and yield of leaf cabbage (kalian or kale) (*Brassica oleracea* var. *acephala*).

MATERIALS AND METHODS

This research was conducted in Kaniti Village, Subdistrict of Kupang Tengah, District of Kupang, East Nusa Tenggara Province, Indonesia from January to October 2019. The location of the research was dominated by alfisol. Materials of the research were fishbone flour and tofu slurry, leaf cabbage, etc.

The factorial research was designed on Randomized Complete Block Design (RCBD) with three replications. Variables of the research were kinds of organic waste (fishbone flour and tofu slurry), and various dosage of organic waste (0, 300, 400, and 500 g polybag⁻¹). Before planting, three soil samples were collected at 0-20 cm soil depth from each replicate area by using an auger and then these samples were mixed with each other to form a composite sample for each treatment. Each soil sample was mixed, then four replications were taken for measurements and analysis and the average readings were recorded. Also at the end of the experiment soil samples were collected in the same manner, as before planting, but replicates of each treatment were not mixed with each other, and analysis was done with four replicate samples, then average readings were recorded. Chemical characteristics of alfisol were measured before treatment and after harvest. Meanwhile, fresh dry weight of leaf cabbage was measured after harvesting.

Parameters measurements were total nitrogen by Kjeldahl method (AOAC 1975), available phosphate by Olsen method (Olsen 1954), exchangeable potassium by

Table 1. The effect of fishbone flour and tofu slurry on total nitrogen content of alfisol

Type of organic waste (T)	Total N (%)	Duncan range	Freq.
T1 (Fishbone flour)	0.25 a	0.05	2
T2 (Tofu slurry)	0.30 b	0.05	3

Note: Numbers followed by the same letter on the same column are not significantly different at Duncan Multiple Range Test of 5% level

Flame Photometer method (Hanway and Heidal 1969), and fresh dry weight of leaf cabbage.

All data measured were analyzed by Analysis of Variant (ANOVA) followed by Duncan Multiple Range Test (DMRT) 5% level.

RESULTS AND DISCUSSION

Total nitrogen

Result of the research showed that there were no interaction effects of kinds of organic waste and various dosages of organic waste on total nitrogen of alfisol. However, each of the single factors of kind of waste and dosage significantly affected total nitrogen of alfisol. Post hoc DMRT at 5% level showed that tofu slurry contained a higher content of total nitrogen than fishbone flour (Table 1). Meanwhile, application of both fertilizer by 400 and 500 g polybag⁻¹ resulted in a higher total nitrogen content as compared to application of fertilizer at 0 and 300 g polybag⁻¹.

This result showed that higher content of total nitrogen applied by tofu slurry was mainly due to organic waste of tofu slurry content was 2.72 % of total nitrogen while fishbone flour contained 2.04% of total nitrogen. Moreover, total nitrogen content of alfisol was 0.2 % (categorized low) before being treated by organic waste. As a result, by application both of organic waste, total nitrogen of alfisol increased significantly. Similarly, results were reported by some studies (Broadbent 1970; Banik 1982; Soetedjo 2018; 2019) that increased total nitrogen of soil depends strongly on nutrient content on the early condition of the soil. Nutrients content of the soil will be increased gradually by application of organic fertilizer if its nutrients were low. Moreover, an increase in nitrogen content at 500 g polybag⁻¹ of organic waste might increase number of microorganisms, improve soil microbacteria activities, improve soil porosity, decrease soil colloid, and improve cation exchangeable capacity. A number of studies (Granatstein et al. 1987; Soetedjo 2008; Beja et al. 2015) reported that increase in number and activities of soil microorganisms as result of various soil management could improve the availability of some nutrients (N, P, and K).

Available phosphor (ppm)

There was no interaction effect of kinds of organic waste and various dosages of organic waste on available phosphor of alfisol. However, the single factor treatment affected significantly the available phosphor of alfisol.

Table 2. The effect of various dosages of organic waste (fishbone flour and tofu slurry) on total nitrogen content of alfisol

Dosage of organic waste (T)	Total N (%)	Duncan range	Freq.
K0 (0 g polybag ⁻¹)	0.04 a	0.06	2
K1 (300 g polybag ⁻¹)	0.30 b	0.07	3
K2 (400 g polybag ⁻¹)	0.35 bc	0.07	4
K3 (500 g polybag ⁻¹)	0.41 c		

Note: Numbers followed by the same letter on the same column are not significantly different at Duncan Multiple Range Test of 5% level

Result of the research showed that tofu slurry resulted in a higher available phosphorus of alfisol than fishbone flour (Table 3). This was mainly due to a moderate available phosphorus content before study affected the ability of the soil to bind and release phosphorus to exchangeable complex of the soil. It seems that low content of available phosphorus of tofu slurry may greatly improve as compared to high content of available phosphorus of fishbone flour. Moreover, it showed that increasing dosage of organic waste up to 500 g polybag⁻¹ affected significantly the available phosphorus content of alfisol as compared to no application of organic waste (Table 4).

Higher content of available phosphorus of tofu slurry as compared to fishbone was mainly due to tofu slurry was likely easier to decompose than fishbone flour. The increasing available phosphorus of alfisol after being treated by the application of various dosages of organic waste was mainly due to decomposition process of organic waste produce humic and fluvic acids which bond tightly by amorph fraction becomes available to the soil (Chauhan et al. 1979; Mulyaningsih 2013; Amtiran 2017; Soetedjo 2018). Increasing available phosphorus might result in increasing source of energy for soil microorganisms to improve their activities in decomposing soil organic matter such as fishbone flour and tofu slurry. Some studies reported that soil microorganism activities increase gradually on well soil aeration, suitable pH, enough some nutrients, enough oxygen, and well available of carbon as source of their energy (Granatstein 1987; Hassink 1994). Some studies showed that there is a positive correlation between improving soil microorganisms and improving some nutrient availability (N, P, and K) (Hassink 1994; Fitri 2011).

Exchangeable potassium

Result of the research showed that interaction of kinds of organic waste and various dosages of organic waste did not significantly affect the exchangeable potassium of alfisol. Similarly, the same results hold for the single factor of kinds of organic waste. However, the single factor dosage of organic waste affected significantly the exchangeable potassium of alfisol. Result of Duncan Multiple Range Test (Table 5) showed that application of 400 - 500 polybag⁻¹ of organic waste produced the highest exchangeable potassium of alfisol. This was mainly due to some of potassium being lost as a result of characteristics

of potassium that moveable easily, some of this loss occurs during plant watering and some are lost through absorption by the plant. These processes significantly decreased soil exchangeable potassium. Therefore, increasing the dosage of organic waste of fishbone flour and tofu slurry might improve the availability and exchangeability of potassium on the soil (Novianantya 2016; Soetedjo 2019).

Moreover, increasing the availability of potassium is likely related to increases in nitrogen. Some studies showed that nitrogen acts synergistically with potassium, but it has an antagonistic action with phosphorus. Some studies showed that organic fertilizer could improve availability of soil microorganisms and could improve the availability of nutrients such as nitrogen, phosphorus, and potassium (Mertikawati et al. 2012; Zannah et al. 2014).

Fresh dry weight of leaf cabbage

Result of the research showed that interaction of kinds of organic waste and various dosage of organic waste and single factor treatment of kinds of organic waste did not affect significantly the fresh dry weight of leaf cabbage. These conditions significantly related to soil nutrient content in which most of the result showed that nitrogen, phosphorus, and potassium were not affected significantly by those treatments. However, the single factor of various dosage of organic waste affected significantly the fresh dry weight of leaf cabbage. This was mainly due to application of organic waste improve significantly nitrogen, phosphorus, and potassium of alfisol that are required to support growth and yield by kalia (Tables 1, 2, 3, 4, and 5).

A number of studies as reported by Soetedjo (2018; 2019) showed that improvement of total nitrogen content, available phosphorus, available potassium, C organic content, and pH resulted in improved yield of mungbean. Increasing total nitrogen content improved the growth and development during vegetative stage of plant as a result plant biomass might increase gradually. Meanwhile, increasing the availability of phosphorus stimulates development of plant to be more vigorous and might improve metabolism processes of plant along with increasing nitrogen content. Improving availability of potassium might improve resistance of plant to pests and diseases. Finally, improvement of metabolism processes supported by the availability of nutrients might affect a better yield of plant.

Table 3. The effect of fishbone flour and tofu slurry on available phosphorus content of alfisol

Type of organic waste (T)	Available P (ppm)	Duncan range	Freq.
T1 (Fishbone flour)	52.35 a	29.67	2
T2 (Tofu slurry)	79.40 b	31.14	3

Note: Numbers followed by the same letter on the same column are not significantly different at Duncan Multiple Range Test of 5% level

Table 4. The effect of various dosages of organic waste (fishbone flour and tofu slurry) on available phosphorus content of alfisol

Dosage of organic waste (T)	Available P (ppm)	Duncan range	Freq.
K0 (0 g polybag ⁻¹)	15.65 a	29.67	2
K1 (300 g polybag ⁻¹)	59.04 b	31.14	3
K2 (400 g polybag ⁻¹)	79.80 bc	32.03	4
K3 (500 g polybag ⁻¹)	109.02 c		

Note: Numbers followed by the same letter on the same column are not significantly different at Duncan Multiple Range Test of 5% level

Table 5. The effect of various dosages of organic waste (fishbone flour and tofu slurry) to exchangeable potassium content of alfisol

Dosage of organic waste (T)	Exchangeable potassium (me/100 g)	Duncan range	Freq.
K0 (0 g polybag ⁻¹)	0.48 a	0.24	2
K1 (300 g polybag ⁻¹)	0.74 b	0.25	3
K2 (400 g polybag ⁻¹)	0.88 bc	0.25	4
K3 (500 g polybag ⁻¹)	1.08 c		

Note: Numbers followed by the same letter on the same column are not significantly different at Duncan Multiple Range Test of 5% level

In conclusion, there was no interaction effect of kinds of organic waste and various dosages of organic waste on all observed parameters. However, each single factor treatment significantly affected total nitrogen content, available phosphorus, exchangeable potassium, and fresh dry weight of leaf cabbage. Tofu slurry significantly improved all nutrient content as compared to those of fishbone flour. Application of both organic waste (tofu slurry and fishbone flour) up to 500 g polybag⁻¹ of organic waste result in higher nutrient content compared to no application of organic waste. Similarly, application 500 g polybag⁻¹ of organic waste result in a higher fresh dry weight of leaf cabbage by 1.08 g.

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