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Effect of Nutrient Enriched Organic Liquid Fertilizers on Growth of *Albemonchus esculentus*

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Abstract

Considerable amounts of fish and fruit wastes are produced on a daily basis due to the fish processing and postharvest handling of fruits. The proper management of organic waste by converting into valuable resources is of utmost importance, in order to reduce their adverse impacts on the environment. This study was aimed to assess the utilizing potential of fish waste hydrolyzed with fruit wastes of papaw *(Carica papaya)* and pineapple (*Ananas comosus)* to enhance the nitrogen content of organic liquid fertilizers. Ripe fruit peel + crown leaves of *A. comosus*, ripe fruit peel + leaves of *C. papaya* and (1:1w/w) combination were used to extract the protease enzymes bromelain, papain and both enzymes respectively. They were mixed separately with 400 g of powdered fish waste and incubated for two days at room temperature. Six different fertilizer combinations were prepared by mixing enzymatically-digested fish waste with eight-week decomposed plant leaves and immature stems of *Tithonia diversifolia, Mikania scandens, Chromolaena odorata* and *Gliricidia sepium* with coconut husk ash and allowed to decompose for another two weeks. Nutrients of the resulting fertilizers were analyzed. *Albemonchus esculentus* plants were foliar sprayed twice a week for three months. Results revealed that, the nitrogen content was higher in fertilizers enriched with fish waste hydrolyzed by papain (0.49%), bromelain (0.38%) and the mixture of both enzymes (0.35%) compared to the control (0.30%). Fish waste hydrolyzed with papain recorded the highest phosphorus (0.05%), potassium (0.34%), calcium (0.26%) and magnesium (0.04%) levels. Fish waste hydrolyzed with papain enzymes recorded the highest number of leaves (16.0±0.8), shoot height (55.9±0.5 cm) and number of pods (19.5±0.5), fruit weight (33.1±1.4 g) followed by the bromelain enzyme and the mixture of both enzymes for the growth of *A. esculentus.* Findings of this study recommend the use of the above organic waste in production of organic liquid fertilizers which would in turn be a low-cost and an eco-friendly alternative for the chemical fertilizers while helping for the sustainable nutrient management and recycle of wastes.

Keywords

Fish Waste, Fruit Waste, Liquid Organic Fertilizers, Protease Enzymes, *Albemonchus esculentus, Carica papaya, Ananas comosus*

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1. Introduction

Application of chemical fertilizers is the most popular agronomic practice that significantly contribute to improve productivity and increase crop yield [40]. However, prolonged and excessive use of chemical fertilizers lead i.e. loss of soil fertility, increased soil acidity and a rapid deterioration of soil and groundwater [30], induce eutrophication, accumulate heavy metals in soil and plants and indirectly increase the crop vulnerability to pests and diseases, reduce soil productivity [19, 33] break the natural eco-system balance by destroying soil inherent microflora -

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fauna and create detrimental effects on the human health [26, 12]. With the emerging concern for environmental protection and sustainability, organic fertilizers have currently become a popular alternative to chemical fertilizers. Organic fertilizers vary widely in form and nutrient content based on the raw materials, method of production and environmental conditions [11]. Among different organic fertilizers, foliar application of organic fertilizers is a new strategy in modern crop management which involves in increasing the nutrient-use efficiency by direct nutrient absorption through the plant foliage. It is effective under lower soil health conditions i.e. limitations or deficiencies of the availability of soil applied nutrients, high nutrient leaching and inadequate root growth due to hindrance of the soil [8]. Foliar application is an environmental friendly and effective fertilization method that has a great potential to alleviate the soil and water pollution problems associated with excessive soil application of fertilizers i.e. nutrient runoff, eutrophication, contamination of the groundwater and soil acidity [16, 25].

Liquid organic fertilizers are made with different organic waste materials including either plant or animal based crop residues, green manures, animal manures, household waste and food waste [23] and provide soluble and easily available nutrients to the crops. Moreover, large quantities of weeds and plant residues that are commonly available in agricultural fields have the great potential to utilize as nutrient-rich sources in formulating compost and vermi-compost [35]. Utilization of weeds in this manner provides an innovative agronomic practice that minimizes the high cost of weed management using mechanical weeding or herbicide application. Among different weeds and green manure in coconut plantations in Sri Lanka, *G. sepium, T. diversifolia* and *Puereria phasioloides* have proven high nitrogen, potassium, organic carbon and worm growth in vermi-compost production [34]. Moreover, coconut husk ash is a good source of potassium, phosphorus and magnesium majorly with many other micronutrients. Coconut husk ash is an excellent mineral fertilizer for immature coconut hybrids to provide potassium [4]. In addition to the plant based materials, other potential organic waste also provide high nutrient which are useful to formulate high-quality organic liquid fertilizers. Among them, fish waste which is produced in large quantities due to high demand for fish consumption creates an additional burden on waste management [18]. Therefore, large amount of unutilized fish waste consists of heads, tails, skin, gut, fins and frames are being produced on a daily basis due to the fish processing industries including stunning, grading, slime removal, de-heading, scaling, gutting, cutting of fins, slicing into steaks, filleting and meat bone separation [29]. Fish waste is utilized for producing fish meal, fish sauces and many other various value-added products such as proteins, oils, amino acids, minerals, enzymes, collagen and gelatin [9]. However, the majority of the fish waste is not been utilized and disposed into the sea, dumped by landfilling or incinerate creating additional large negative impacts on the environment without considering their remaining nutrient values. Therefore, there is an urgent requirement of searching ecologically acceptable ways of re-utilization methods of fish waste. In this view, fish waste has a great potential of utilizing in agricultural purposes as they are capable of promoting plant growth and yield providing 4-10% of N, 3-9% of P_2O_5 and 0.3-1.5% of K_2O [18, 6]. Moreover, fish waste is a quick-acting organic manure that proved great success in vegetable production systems when incorporated as a soil amendment [10]. Liquid fertilizer formulated with fish waste mixed with *T. diversifolia* has shown a significant growth enhancement of *A. esculentus* after foliar application [15]. Moreover, two weeks fermented liquid fish waste including heads, guts, fins and bones also showed significantly higher growth performances of *Solanum melongena* [3]. They revealed that fish waste had significantly increased the leaf area by enhancing the cell division and elongation leading high crop yield due to their high nitrogen content than plants treated with urea. Furthermore, liquid fish emulsion made from byproducts of the fish industry also resulted in higher growth performances for lawns and leafy green vegetables due to the presence of high nitrogen levels [14].

Similarly, large amounts of fruit wastes produced during harvesting, processing, storing or post-harvest handling are widely available and accumulate in market complexes and agro-industrial yards without having any commercial value. These residues also cause serious environmental problems due to the high costs of transportation and the limitation of available lands for disposal. Thus, fruit waste are used for extraction of enzymes and different secondarily low-cost raw materials i.e. ethanol, phenolic anti-oxidants, organic acids, biogas and fibers [36]. Among them, proteolytic enzymes present in fruits or fruit wastes are widely used for hydrolysis of different protein substrates and tenderization of meat and fish since ancient times [17]. During the enzymatic degradation, the peptide bonds in protein substrates are hydrolyzed to shorter peptides or amino acids resulting in higher collagen solubility [31]. Ketnawa and Rawdkuen (2011) [17], investigated the successful tenderizing effects of bromelain extract obtained from pineapple fruit peel on uniform-sized chunks of beef, chicken and squid. Similarly, papain and bromelain extracted from papaya latex and bromelain from pineapple stem were successful for the hydrolysis of fish frames [13]. In addition to hydrolysis of fish and meat waste, the influence of papain over hydrolysis of aqueous extracts of cauliflower, tomato leaves and whole tomato plant residue as liquid organic fertilizers were evaluated by Gioseffi *et al*, (2009) [9]. Results suggested that the addition of papain (400 mg L^{-1}) had increased the amount of total nitrogen and mineral nitrogen in all the extracts compared to the control. Among the available literature, few attempts have been made to utilize solely the selected weeds or fish waste as a nutrient-rich organic source in formulating different organic fertilizers [18] and fewer studies have utilized fruit waste with fish waste [13] and fish waste with selected weeds [15]. However, there is no any record on the utilization of pre-digested fish waste using fruit waste of *A. comosus* and *C. papaya* mixed with the selected weed plant materials.

Palm oil mill effluent (POME) is another potential by-product of palm oil extraction that is very difficult to manage as it produces in large volumes at a time. Therefore, as a low-cost and the easiest method of disposal, untreated or partially treated POME is being discharged into nearby water reservoirs, rivers or lands by industries [21]. However, that causes detrimental effects on both soil and aquatic system. Therefore, studying beneficial utilization methods of POME as a food for pigs, poultry and fish; energy source; fertilizer and fermentation media [42] is important towards a sustainable and economical waste management system.

As a novel approach, the aim of this study was to assess the potential of utilizing *A. comosus* and C. *papaya* fruit waste for the hydrolysis of fish waste that combined with the selected organic waste i.e. weed plant material of *T. diversifolia, C. odorata* and *M. scandens* with a green manure *G. sepium*; POME; coconut husk ash to formulate liquid organic fertilizers. Accordingly, proper processing and recycling of organic waste into resources that provide nutrients in agriculture can effectively reduce the environmental impacts.

2 Materials and Methods

2.1. Collection of Potential Organic Wastes and Weeds for Enriching the Organic Liquid Fertilizer

As potential organic wastes, coconut husk ash and powdered fish waste were collected from a private coconut state at Gamapaha and Central Fish Marketing Complex at Peliyagoda Sri Lanka respectively. Fruit waste of *C. papaya* and *A. comosus* were collected from the Central Fruit and Vegetable market at Kiribathgoda and palm oil mill effluent (POME) was collected at a palm oil mill located in Baduraliya, Sri Lanka. Fresh leaves and tender shoots of the highly abundant common weeds i.e. *T. diversifolia, C. odorata* and *M. scandens* with a green manure *G. sepium* were collected from unpolluted and non-cultivated areas, away from the roadsides from Gampaha area in Sri Lanka.

2.2. Determination of Physiochemical Characteristics of Selected Organic Wastes

Coconut husk ash was produced by complete burning of the dried mature coconut husk in an open fire in an earth pit. Collected plant materials of *G. sepium* was washed twice with distilled water to remove any contaminants or dust particles and air dried at room temperature and oven dried at 80°C for constant weight. The dried plant samples were homogenized using a cleaned mortar and pestle and sieved through a 2mm mesh. Subsequently, the sieved plant samples, coconut husk ash, powdered fish waste and palm oil mill effluents were checked for their initial nutrient contents. Total nitrogen contents in samples were determined by the Kjeldahl method [22]. Tri-acid digested samples with HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 9:4:1 were analyzed for phosphorus using vanadium-phosphomolybdate colorimetric method and potassium, calcium, magnesium, copper, iron, zinc and manganese using Atomic Absorption Spectrophotometer (Varian Spectra A-110) using the protocols described by [22].

2.3. Analysis of Physicochemical Properties of POME Samples

POME was analyzed for the physicochemical properties i.e. pH, electrical conductivity, total dissolved solids and dissolved oxygen using the HQ 40-d multi-parameter. Furthermore, the initial oil and grease contents were determined according to the solvent extraction method using n-hexane as the solvent followed by the Soxhlet extraction technique [5]. Furthermore, 500ml of raw effluent was allowed to settle down for 48 hours in a closed 500 ml flask and in the following day, the settled liquid portion was carefully filtered using a muslin cloth without disturbing the sediment part. This was followed with four replicates and oil and grease content of two layers were determined and the least oil and grease containing layer was selected for formulation of organic fertilizers.

2.4. Decomposition of Plant Materials and Formulation of Organic Liquid Fertilizers

Two kilograms of fresh leaves and immature twigs of the selected plant species i.e. *T. diversifolia, M. scandens* and *C. odorata* were washed thoroughly with distilled water and chopped into small pieces separately. Plant materials of each species were mixed with 50g of topsoil collected from the Botanical Garden of the University of Kelaniya, Sri Lanka with 3L of distilled water in a 25L plastic containers separately and allowed to decompose for two weeks with daily stirring. Thereafter, the additional components i.e. powdered fish waste (200g), filtered palm oil mill effluent (1L) and distilled water (1L) were mixed with partially decomposed plant materials and allowed to further decompose for six weeks. During the decomposition period, containers were kept covered with 2mm nylon mesh and daily aerated for two hours using an aerator to accelerate the decomposition. Fifty grams of coconut husk ash was added to all four fertilizer combinations (Table 1) and final filtrates were subjected to macro and micro-nutrient analysis as mentioned above.

Table 1. The composition of the formulated liquid fertilizers.

Fertilizer	Common components	Additional components
FA1	*T. diversifolia* (2kg)	Distilled water (4L)
FA2	*M. scandens* (2kg)	Powdered fish waste (200g) + distilled water (4L)
FA3	*C. odorata* (2kg)	Filtered POME (1L) + distilled water (3L)
FA4	Topsoil (150g) + Coconut husk ash (50g)	Powdered fish waste (200g) + filtered POME (1L) + distilled water (3L)

2.5. Application of Selected Fertilizers on *A. esculentus*

Plant trials were conducted in the plant house of the Botanical Garden at the University of Kelaniya, Sri Lanka (6°58'26.4"N, 79°54'54.72"E) from April to July in 2016. During the experimental period, the average monthly temperature and relative humidity ranged from 28 - 32°C and 65% respectively. A completely randomized block design with six treatments and five replicates was used. The treatments were as follows; the highest nutrient containing four fertilizer combinations FA1, FA2, FA3 and FA4, distilled water as the negative control (C) and commercially available liquid organic fertilizer as the positive control (M). Seeds of *A. esculentus* (Cultivar: Haritha) were obtained from Department of Agriculture, Peradeniya, Sri Lanka and three seeds were sawn in each pot (diameter of 16 cm x height of 18cm) containing sieved and solarized normal garden soil and three weeks after the germination, one healthy plant per pot was maintained. Five milliliters of 1:50 diluted formulated liquid fertilizers were sprayed on foliar parts on each plant twice a week for a one month followed by spaying 15ml of fertilizers for the next two months on each plant as the plant grow. As growth parameters i.e. the number of leaves, leaf area and shoot height were recorded and the pods were harvested at maturity and pod weight and length were measured.

2.6. Qualitative Determination of the Presence of Proteolytic Enzymes in *C. papaya* and *A. comosus* [38]

Protease enzyme solution from *C. papaya* was prepared using the ripe fruit peel + leaves in 1:1 ratio (w/w) by mixing 5g from each. Those were cut into small pieces and ground using a ceramic motor and pestle using 25ml with distilled water (1:5w/w). The extract was filtered through a muslin cloth and centrifuged at 5,000rpm for 15mins to obtain the clear supernatant rich in papain enzyme. Following the same procedure, *A. comosus* fruit waste including ripe fruit peel + crown leaves 5g from each were used to obtain an extract rich in bromelain enzyme. The presence of the proteolytic enzyme in the prepared enzyme solutions was tested, using casein as the substrate [2]. Therefore, 10 ml fresh-milk of 20% (v/v) was used as a casein substrate and pH of the milk solution was adjusted with diluted acetic acid (0.01%). Prepared enzyme solution (0.50mL) was added to the milk sample and its temperature was maintained at 37°C and observed for the milk coagulation. The control test was carried out incubating a milk sample without any enzyme solution.

2.7. Improvement of the Selected Organic Liquid Fertilizer

2.7.1. Pre-enzymatic Digestion of Fish Waste Using Fruit Wastes and Leaves of *C. papaya* and *A. comosus*

Powdered fish waste 400g was taken to a 10L plastic container and homogenized well with 1200mL of distilled water separately. Protease enzyme solution from *C. papaya* was prepared using the ripe fruit peel + leaves in 1:1 ratio (w/w) by mixing 80g from each. Those were cut into small pieces and ground using a ceramic motor and pestle, filtered and diluted to 400ml with distilled water to obtain an extract rich with papain enzyme. In the same way, *A. comosus* fruit waste including fruit peel + crown leaves 80g from each were crushed, filtered and diluted to 400ml with distilled water to obtain an extract rich in bromelain enzyme. Then 400mL of each enzyme solution was mixed separately with 1200mL of prepared fish waste samples to make fish waste + *C. papaya* enzyme extract, fish waste + *A. comosus* enzyme extract and fish waste + *C. papaya* + *A. comosus* enzymes (1:1) extract.

2.7.2. Mixing of the Digested Fish Waste with Decomposed Plant Materials

Fish waste and enzyme mixtures that incubated two days under room temperature were mixed separately with eight weeks-decomposed plant materials (Table 2). Following the above mentioned same procedure, all the samples were filtered and the filtrates were subjected to nutrient analysis.

For each treatment, four replicates were conducted and all the treatments were arranged in a completely randomized design.

Table 2. The composition of the improved liquid fertilizers.

Fertilizer	Common components	Additional components
FB1	*T. diversifolia* (2kg)	Fish waste (400g) + aqueous extract of *C. papaya* (ripe peel + leaves) + Coconut husk ash 50g
FB2	*M. scandens* (2kg)	Fish waste (400g) + aqueous extract of *A. comosus* (ripe peel + crown leaves) + Coconut husk ash 50g
FB3	*C. odorata* (2kg)	Fish waste 400g + (1:1 aqueous extract of *A. comosus* + *C. papaya*) + Coconut husk ash 50g
FB4	*G. sepium* (2kg)	Fish waste 400g + Coconut husk ash 50g
FB5	Topsoil (150g)	Fish waste 400g
FB6	Distilled water (5L)	-

2.8. Application of Improved Fertilizers on *A. esculentus*

Similarly, another plant trial of *A. esculentus* was conducted at the University of Kelaniya, Sri Lanka using a completely randomized block design with six treatments and five replicates. The treatments were the highest nutrient containing four improved fertilizer combinations FB1, FB2, FB3 and FB4, distilled water as the negative control (C) and commercially available liquid organic fertilizer as the positive control (M) and at the end same growth parameters were measured.

2.9. Statistical Analysis

Data on all the nutrient analysis and plant growth performances from the crop trial obtained were analyzed statistically using one-way analysis of variance (ANOVA, $p<0.05$) followed by Tukey's pair-wise multiple comparisons to determine the significant differences between the treatments using IBM SPSS software package (SPSS version 20 for Windows).

3. Results and Discussion

3.1. Identification of Potential Organic Wastes for Enriching the Organic Liquid Fertilizer

Organic materials act as an cost effective and locally available sources of plant nutrients. Among the analyzed organic wastes, coconut husk ash recorded the highest potassium and magnesium contents 9.28±0.02% and 1.01±0.01% respectively (Figure 1). Similarly, Yogarathnam and Silva (1987) [43] stated that coconut husk ash contained the highest potassium content of 20 - 30% K_2O among the different types of organic manure i.e. animal manure, compost, wood ash and paddy husk ash. Fish waste is another potential organic waste that produces in large quantities causing severe environmental issues in discarding due to its unpleasant odor and remaining nutrients. As fish contains high amounts of proteins, fish waste also contains large amounts of nitrogen and many other macro and micronutrients which can be utilized as a soil amendment in agriculture [10]. According to the results, powdered fish waste resulted in significantly the highest nitrogen, phosphorus, potassium, calcium and zinc contents as 7.26±0.16%, 1.27±0.01%, 0.45±0.01%, 4.12±0.10% and 217.3±3.05 mg kg^{-1} respectively (Figure 1 and 2). Similarly, [6] also reported that fish waste contained high nutrient amounts as 4-10% N, 3-9% P_2O_5 and 0.3-1.5% K_2O. Furthermore, the common green manure *G. sepium* also resulted in considerable amounts of nitrogen (3.23±0.01)% next to the powdered fish waste resulted in high nitrogen content with many other macro and micronutrients among the plant-based organic amendments proving the possibility of utilizing in organic fertilizers.

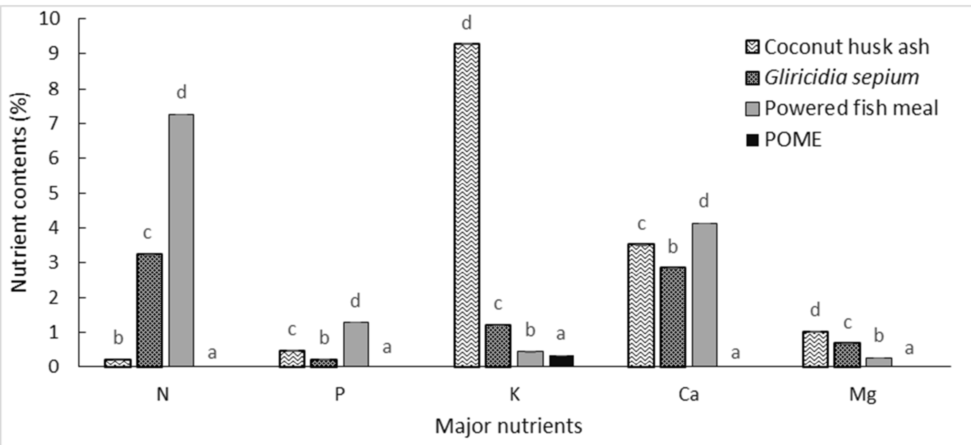

Figure 1. Macronutrient contents of the organic wastes used.

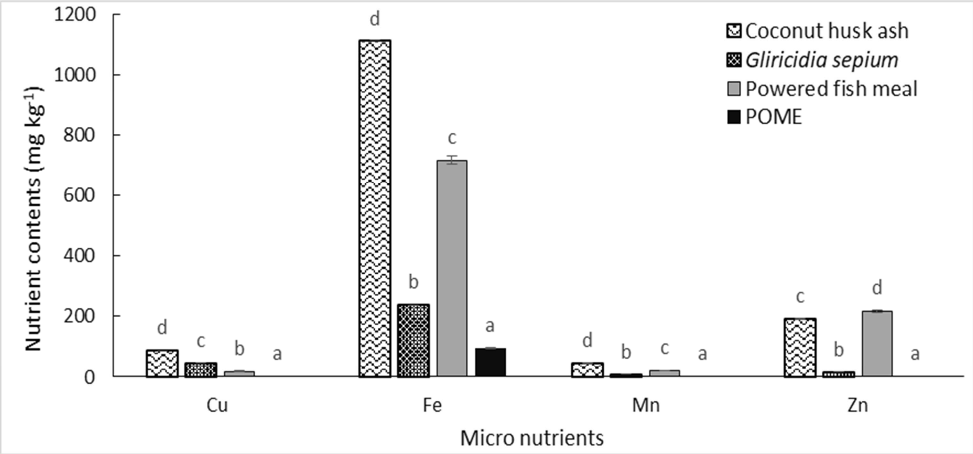

Figure 2. Micro-nutrient contents of the organic wastes used.

Each data point represents the mean of three replicates±standard deviation; Means sharing the same letter (s) in each nutrient are not significantly different at $p<0.05$.

3.2. Potential of Utilizing Palm Oil Mill Effluent (POME) as an Organic Liquid Fertilizer

The palm oil raw effluent recorded the least amount of all macro and micronutrients i.e. nitrogen (0.01±0.00)%, phosphorus (0.02±0.00)% and potassium (0.33±0.01)% among the analyzed the other analyzed organic sources (Figure 1 and 2). It was a thick, viscous and brownish colloidal mixture of water, oil and fine suspended solids with an average low pH value of 4.35±0.01 and comparatively high oil and the grease content of 2973.3±128.6 mg L^{-1} (Table 3). Similarly, Kosit (2011) [20] also reported that the POME typically consisted of pH ranging from 4.0-4.8 with oil and the grease contents of 1300-4700 mg L^{-1}. They further stated that the palm oil raw effluent was a highly polluting effluent containing various forms of organic and inorganic suspended solids which causes many environmental issues and pollution due to improper disposal. Furthermore, Madaki and Seng (2013) [21] pointed out that, POME cannot be discharged without being treated due to the presence of residual oil and its acidic nature. Therefore, POME sample was allowed to sediment for 48 hours and filtered to reduce its oil and grease content. It resulted in 380.3±20.0mg L^{-1} of lower oil and grease content in the top filtrate layer whereas, 3660.0±130.5mg/L in the bottom layer as most of the oil residues were trapped with fibrous solid residues in the bottom layer (Table 4). This observation is in accordance with [39] who reported that, the number of residual oil droplets and solid particles in the bottom layer was the highest than the middle and the top layer in-order, studying the microscopic view of 1 hr settled POME. They further stated that the fibrous particles are normally larger than oil droplets and settled to the bottom faster while oil droplets are trapped inside them as it resulted in increase of the residual oil content from 0.25% to 0.43% and dry matter content from 4.0% to 6.8% in the bottom layer whereas, top layer decreased both factors from 2.0% to 1.5% and 0.15% to 0.05% respectively. Moreover, Ahamad *et al*., (2005) [1] also reported that fibrous debris has the ability to high oil absorbi04due to its oleophilic nature. Moreover, POME resulted in comparatively low nutrient content compared to the other potential sources, however it showed higher potassium content and the filtered top layer resulted in comparatively lower amounts of nutrients compared to the non-filtrated sample except for the potassium (3660±28.3mg L^{-1}) and magnesium content (174±2.5mg L^{-1}) (Table 5).

Table 3. Physicochemical properties of POME sample.

Parameter	Average value (±SD)
pH	4.35±0.01
Conductivity (mS/cm)	8.91±0.02
Total dissolved solid (g/L)	4.81±0.03
Oil and grease content (mg/L)	2973.3±128.6

Table 4. Oil and grease contents of the two layers of POME after 48 hrs of settlement.

Sample	Oil and grease content (mg/L)
Top filtrate layer	380.3±20.0
Bottom sediment layer	3660.0±130.5

Table 5. Nutrient content of different POME samples (Mean±SD).

Nutrient	Sample	
contents (mg/L)	Raw effluent	Filtrate of the raw effluent
N	100.0±0.0	65.0±0.0
P	164.5±2.1	128.5±0.7
K	3340.0±84.9	3660±28.3
Ca	132.0±0.0	89.0±4.2
Mg	174.0±0.0	174±2.5
Cu	1.8±0.1	0.85±0.1
Fe	95.6±0.8	79.0±0.6
Mn	3.5±0.1	2.8±0.1
Zn	2.8±0.0	2.2±0.0

3.3. Qualitative Determination of the Presence of Proteolytic Enzymes in *C. papaya* and *A. Comosus*

When all the prepared enzyme solutions were added to the milk solution, all the treatments showed positive results of coagulations with milk. The negative control which contained only the milk solution did not given any observation of milk coagulation as it lacks the enzyme source. This simple test confirmed the presence of proteolytic enzymes in *C. papaya* and *A. comosus* plant extracts.

3.4. Nutrient Contents of the Formulated Liquid Fertilizers

Decomposed or processed organic wastes provide an excellent source of nutrients that is suitable for organic farming while facilitating the successful nutrient recycling towards a sustainable waste management [9]. Among the formulated liquid fertilizers, FA2 (*T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + fish waste + coconut husk ash) combination recorded the highest N, P, K, Ca and Mg contents (2166.7±57.7, 270±5.0, 4923.7±63.9, 292.0±2.6 and 258.6±3.2mg L^{-1} respectively) followed by the FA4 (*T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + fish waste + filtered POME + coconut husk ash) as they contained fish waste as the main nitrogen source and coconut husk ash as the main potassium source with selected invasive plant materials (Table 6). Moreover, application of coconut husk ash also provides Mg and P to enhance the crop growth [12]. The least amount of N, K, Ca and Mg were recorded in FA1 (*T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + coconut husk ash) as it was formulated without adding any fish waste. Similarly, *T. diversifolia* and *C. odorata* contained a higher amount of nitrogen among the plant-based weed materials and that was not sufficient alone as a fertilizer compared to other compositions [24]. Moreover, topsoil with plant litter was added as an inoculant of favorable soil fungal and bacterial communities that facilitate the successful organic matter degradation by breaking down of large organic molecules to small molecules [26]. Furthermore, there were no significant differences of nitrogen, potassium, calcium and magnesium contents between the FA1 fertilizer and FA3 fertilizer which had additionally incorporated with the filtered raw effluent proving that POME was not involved in significantly increase the nutrient contents of the composition.

Table 6. Macro-nutrient contents of the formulated liquid fertilizers.

Fertilizer	Macronutrient contents (mg/L)				
	N	P	K	Ca	Mg
FA1	896.7±5.8^{a}	105.0±5.0^{b}	4433.3±57.7^{b}	90.7±4.0^{a}	179.0±3.6^{a}
FA2	2166.7±57.7^{c}	270.0±5.0^{d}	4923.6±63.8^{c}	292.0±2.6^{b}	258.6±3.2^{c}
FA3	903.3±5.7^{a}	93.0±2.6^{a}	4643.3±37.8^{b}	98.3±1.5^{a}	182.0±3.0^{a}
FP4	1898.3±2.9^{b}	217.3±6.4^{c}	4871.7±16.1^{c}	291.0±1.0^{b}	206.7±3.1^{b}
Standard	2422.0±19.3^{d}	92.0±0.0^{a}	3058.7±165.0^{a}	300.1±3.7^{b}	261.0±8.0^{c}

Each data point represents the mean of three replicates±standard deviation; Means sharing the same letter in each column are not significantly different at $p<0.05$.

FA1; *T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + coconut husk ash, FA2; *T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + powdered fish waste + coconut husk ash, FA3; *T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + filtered POME + coconut husk ash, FA4; *T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + filtered POME + powdered fish waste + coconut husk ash.

Liquid organic fertilizer is a successful method of supplying major nutrients; nitrogen, phosphorus and potassium during the critical growth stages, whilst supplying the secondary nutrients (magnesium, calcium, sulfur) and micro-nutrients (manganese, zinc, iron, copper, molybdenum and boron) [11]. There were significant differences in plant growth parameters of *A. esculentus* after the twelve weeks of foliar fertilizer application (Table 6). Similar results were obtained by the study of [26], proving that the growth and yield of rice have increased significantly by liquid organic fertilizers. The highest average shoots height, *i.e.* 56.96±2.2cm of *A. esculentus* was obtained in plants treated with the FA2 fertilizer followed by FA1 (52.12±1.1cm) and it was significantly higher compared to the commercially available standard fertilizer. The lowest shoot height was recorded for the control (43.5±1.1cm). The highest number of leaves per plant of *A. esculentus* also obtained for the FA2 (14.0±0.1) followed by the standard (13.0±0.4) showing significant differences whereas, the control possessed the minimum (9.0±0.4). Similarly, the number of fruits per plant also followed the same trend whereas, it showed no significant difference among FA2 and the standard. With respect to the other growth parameters, i.e. fruit weight and fruit length of *A. esculentus,* the maximum was observed in FA2 fertilizer followed by FA1 and the lowest was recorded in the control (Table 7). The results of the present study are in accordance with the growth performances results of *A. esculentus* [15].

Considering all the growth performances and weighing them according to the parameters studied, the FA1 fertilizer proved to be the best liquid fertilizer for the growth of *A. esculentus.* FA3 fertilizer recorded the minimum growth performances among the checked four formulations it could be due to the poor nutrient content, low pH condition and remaining oil and grease contents in the filtered palm oil mill effluent. Although the fertilizer combination FA4 that contained filtered palm oil mill effluent with fish waste showed the second highest nutrient contents next to the FA2 fertilizer and its plant growth performances were lowered and not significant compared to FA1 fertilizer. This can be due to the remaining oil and grease contents in POME and presence of low nutrient content in the filtered sample. Therefore, further improvements are needed prior being applied for agricultural purposes and even it was deselected as it is not available organic waste throughout the country as palm gardens are located in the limited areas in southern part of the country. Therefore, FA2 fertilizer combination was selected for the improvement.

Table 7. Effect of formulated liquid organic fertilizers on the growth performance of *A. esculentus.*

Growth parameter	FA1	FA2	FA3	FA4	Control	Standard
Shoot height (cm)	52.1±1.1^{c}	56.9±2.2^{d}	48.5±1.2^{b}	51.1±1.1^{c}	43.5±1.1^{a}	51.1±0.8^{c}
No. of leaves per plant	11±0.8^{b}	14±0.1^{d}	10±0.7^{b}	12±0.4^{bc}	9±0.4^{a}	13±0.4^{c}
No. of fruits per plant	11.0±0.8^{b}	14.0±0.5^{c}	10.0±0.0^{b}	11.0±0.6^{b}	9±0.4^{a}	14±0.5^{c}
Fruit weight (g)	30.0±0.9^{bc}	33.1±1.4^{d}	26.2±0.4^{b}	29.3±1.7^{c}	21.0±0.5^{a}	30.1±0.9^{c}
Fruit length (cm)	19.4±0.1^{b}	21.5±0.4^{c}	19.7±0.8^{b}	20.3±0.1^{b}	17.4±0.1^{a}	22.0±0.1^{c}

Each data point represents the mean of five replicates±standard deviation; Means sharing the same letter in each row are not significantly different at p<0.05 by Tukey's multiple comparison test.
FA1; *T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + coconut husk ash, FA2; *T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + powdered fish waste + coconut husk ash, FA3; *T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + filtered POME + coconut husk ash, FA4; *T. diversifolia* + *M. scandens* + *C. odorata* + topsoil + filt ered POME + powdered fish waste + coconut husk ash.

3.5. Nutrient Contents of the Improved Liquid Fertilizers

The selected liquid fertilizer was improved adding *G. sepium* and following the enzymatic pre-digestion of fish waste using fruit waste and leaves of *A. comosus* and *C. papaya.* Utilization of protease enzymes catalyzes the mineralization of nitrogen and releases mineral nitrogen from organic compounds presented in waste materials [9]. Enzymatic pre-digestion was followed in order to increase the available nitrogen contents after hydrolyzing with plant-based protein-digesting enzymes [41] as hydrolysis of fish waste without using an enzyme is a slow process [37]. Moreover, the combined digestion of fish waste using fruit waste of *A. comosus* (ripe peel + crown leaves) and *C. papaya* (ripe peel + crown leaves) provide a solution for solid organic waste management as considerable amounts of both fish and fruit waste are produced daily basis due to the fish processing and postharvest handling respectively. Moreover, utilization of decomposed fruits and vegetables in organic liquid fertilizer formation provides hormones and vitamins that are needed for plant growth [20]. Furthermore, proper management and utilization of organic wastes in this manner prevent nutrient losses through runoff and leaching. Among the three enzymatically digested fertilizers FB1, FB2 and FB3 formulated with mixtures of different protease enzymes, majorly with papain, bromelain and a mixture of papain + bromelain resulted in higher nitrogen contents (0.49±0.01, 0.38±0.01, 0.35±0.0% respectively) compared to the undigested fertilizer FB4 (0.30±0.0%). Similarly, Ketnawa and Rawdkuen (2011) [13] have reported both papain and bromelain from papaya latex and pineapple stem respectively were successful for the hydrolysis of fish frames compared to the pancreatin from porcine pancreas. However, among plant-based two enzymes, papain has resulted in the complete hydrolysis. Moreover, FB1 fertilizer which the fish waste was digested with ripe peel and leaves of *C. papaya* resulted in the highest potassium and calcium contents (0.34±0.01 and 0.26±0.0% respectively) whereas the FB6 possessed the minimum potassium and calcium contents (0.21±0.01 and 0.05±0.00 respectively) (Table 8). As shown in the Table 9, FB1 showed the highest iron content (100.2±0.0mg L^{-1}) followed by FB2, FB3 and FB4 which had incorporated with the coconut husk ash. Similarly, application of coconut husk ash has a great potential of supplying K, Mg and P to enhance the crop growth [12]. In addition, FB4 possessed the highest manganese and zinc contents (5.6±0.1, 23.8±0.0mg L^{-1}) followed by FB2 (3.6±0.2mg L^{-1}) and FB3 (15.5±0.0mg L^{-1}) respectively. Moreover, FB4 combination recorded the highest copper content (1.8±0.0mg L^{-1}) whereas the minimum was recorded for FB2 and FB6.

Table 8. Macro-nutrient contents of the formulated liquid fertilizers.

Fertilizers	Macro-nutrient contents (%)				
	N	P	K	Ca	Mg
FB1	0.49±0.01^{d}	0.05±0.0^{c}	0.34±0.00^{d}	0.26±0.0^{f}	0.04±0.0^{a}
FB2	0.38±0.02^{b}	0.01±0.0^{a}	0.29±0.00^{c}	0.09±0.0^{c}	0.03±0.0^{a}
FB3	0.35±0.00^{b}	0.03±0.0^{b}	0.25±0.00^{b}	0.11±0.0^{d}	0.03±0.0^{a}

Fertilizers	Macro-nutrient contents (%)				
	N	P	K	Ca	Mg
FB4	0.30±0.00^{c}	0.08±0.0^{d}	0.24±0.01^{b}	0.15±0.0^{e}	0.05±0.0^{b}
FB5	0.27±0.01^{b}	0.01±0.0^{a}	0.24±0.01^{b}	0.05±0.1^{b}	0.03±0.0^{a}
FB6	0.25±0. 00^{a}	0.01±0.0^{a}	0.22±0.00^{a}	0.06±0.0^{b}	0.04±0.0^{ab}
Standard	0.24±0.0^{a}	0.01±0.0^{a}	0.36±0.02^{e}	0.03± 0.0^{a}	0.03±0.0^{a}

Table 9. Micro-nutrient contents of the formulated liquid fertilizer.

Fertilizers	Micro-nutrient contents (mg L^{-1})			
	Cu	Fe	Mn	Zn
FB1	0.90±0.00^{b}	100.20±0.00^{d}	3.50±0.00^{c}	13.36±0.25^{d}
FB2	0.00±0.00^{a}	98.50±0.12^{d}	3.63±0.64^{c}	1.77±0.12^{a}
FB3	0.80±0.00^{b}	83.10±1.08^{c}	3.26±0.21^{c}	15.50±0.00^{e}
FB4	1.83±0.10^{c}	75.83±4.60^{c}	5.56±0.12^{d}	23.80±0.00^{f}
FB5	0.10±0.00^{a}	35.10±0.36^{b}	1.50±0.00^{b}	3.83±0.35^{b}
FB6	0.00±0.00^{a}	12.43±0.15^{a}	1.96±0.06^{b}	4.37±0.12^{c}
Standard	0.33±0.00^{a}	16.6±0.00^{a}	0.46± 0.02^{a}	3.86±0.03^{a}

Each data point represents the mean of three replicates±standard deviation; Means sharing the same letter in each column are not significantly different at p<0.05.

FB1; *T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil + coconut husk ash + fish waste + aqueous extract of *C. papaya*, FB2; *T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil + coconut husk ash + fish waste + aqueous extract of *A. comosus,* FB3; *T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil + coconut husk ash + fish waste + aqueous extract of *C. papaya* + *A. comosus,* FB4; *T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil + coconut husk ash + fish waste, FB5; *T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil + fish waste; FB6; *T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil.

3.6. Effect of Improved Liquid Fertilizers on the Growth of *A. esculetus*

According to the growth performances of the foliar application of improved fertilizers, fish waste hydrolyzed with papain enzyme (FB1 fertilizer) recorded the highest shoot height (55.9±0.5cm) and number of leaves (16.0±0.8) followed by FB2 fertilizer where fish waste was hydrolyzed with bromelain enzyme. The lowest was recorded for the control. Considering the edible fruit yield, the height number of fruits per plant was recorded in FB1 fertilizer followed by FB2 fertilizer (19.0±0.5 and 18.0±0.1 respectively). Moreover, the highest fruit length and fruit weight also noted in the plants treated with FB1 fertilizer whereas the least was recorded in the control (Table 10). Compared to the solely made enzymes of papain and bromelain rather than the 1:1 mixture, both had resulted in higher nutrient contents and higher plant growth performances. Moreover, all these three combinations have shown more or less same or even higher growth performances than the commercially available liquid fertilizer. It showed that enzymatic pre-digestion of fish waste with fruit wastes of *A. comosus* and *C. papaya* had improved the nutrient contents in all combinations leading for better growth compared to the standard facilitating easily absorbable nutrients.

Table 10. Effect of formulated liquid organic fertilizers on the growth performance of *A. esculentus.*

Growth parameter	FB1	FB2	FB3	FB4	Control	Standard
Shoot height (cm)	55.9±0.5^{d}	54.5±1.0^{c}	51.0±1.2^{b}	50.5±1.6^{b}	45.0±0.5^{a}	52.1±1.4^{b}
No. of leaves per plant	16±0.8^{d}	16±0.5^{d}	15±0.7^{c}	13±1.1^{b}	9±0.5^{a}	14±0.7^{b}
No. of pods per plant	19.0±0.5^{d}	18.0±0.1^{c}	15.0±0.0^{b}	13.0±0.6^{b}	9±0.5^{a}	14±0.5^{b}
Fruit weight (g)	33.1±1.4^{d}	33.0±0.9^{cd}	32.2±0.4^{c}	28.3±1.7^{b}	24.0±0.4^{a}	31.1±0.2^{c}
Fruit length (cm)	23.5±0.2^{c}	22.4±0.5^{b}	22.7±0.1^{b}	21.3±0.1^{b}	17.6±0.4^{a}	22.1±0.0^{b}

Each data point represents the mean of six replicates±standard deviation; Means sharing the same letter in each row are not significantly different at p<0.05 by Tukey's multiple comparison test.

FB1; *T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil + coconut husk ash + fish waste + aqueous extract of *C. papaya*, FB2; *T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil + coconut husk ash + fish waste + aqueous extract of *A. comosus,* FB3; *T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil + coconut husk ash + fish waste + aqueous extract of *C. papaya* + *A. comosus,* FB4; *T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil + coconut husk ash + fish waste.

4. Conclusions

This study revealed that fish waste pre-hydrolyzed with fruit waste of *A. comosus* and *C. papaya* has significantly increased the available nutrient content, mainly the nitrogen content along with the selected weeds of *M. scandens, T. diversifolia, C. odorata* and the green manure *G. sepium* in formulation of liquid organic fertilizers. Among different combinations FB1 fertilizer (*T. diversifolia* + *M. scandens* + *C. odorata* + *G. sepium* + topsoil + coconut husk ash + fish waste + aqueous extract of *C. papaya*) that the fish waste digested with papain resulted in the highest nutrient content and proved to be the best liquid organic fertilizer for the growth of *A. esculantus*. Utilization of fish waste and fruit waste of *C. papaya* and *A. comosus* as sources of plant nutrients in formulation of eco-friendly organic fertilizers will contribute to sustainable waste management and recycling of organic waste. Moreover, utilization of selected weeds will effectively help to control their spread reducing the adverse ecological impacts.

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