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Analysis of NPK in *Camellia sinensis*, *Gliricidia sepium*, and *Musa acuminata* Biomasses for Preparation of an Organic Fertilizers Formula for Young Tea Plants (*Musa acuminata*) and Studying of Their Nutrient Release Capacity in the Biodegradation Process

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Abstract

This study investigates the nitrogen (N), phosphorous (P), and potassium (K) contents in raw biomasses of *Camellia sinensis*, *Gliricidia sepium*, and *Musa acuminata.* Therein, the highest N and P content was seen in *Camellia sinensis* 116.80 ± 0.08 mg and 66.00 ± 0.14 mg respectively. The highest K content (106.80 ± 0.04 mg) was observed in *Musa acuminata.* Next, all three types of plant materials were allowed to decompose in water for 3 weeks, and a sample from each was analyzed for NPK after the 1st, 2nd, and 3rd week during decomposition. A significant increase in the release of N, P, and K by the *Camellia sinensis* to water ($P < 0.05$) was observed during the decay. However, the release of K by *Gliricidia sepium* and N by *Musa acuminate were not* significantly changed ($P > 0.05$) over time. The ratio for N:P:K was calculated for raw biomass samples and decomposed samples to find the best fitting N:P:K ratio to apply to young tea plants as organic fertilizers. In addition to that, the microbial insight of these organic compounds was analyzed by observing how microbial population increased with decomposition by the enumeration of the total microbial count. A considerable increment in total microbial count was observed up to 3.28×10^6, 1.21×10^{10}, 2.18×10^8, and 6.49×10^7 CFU/ml for *Camellia sinensis*, *Gliricidia sepium*, *Musa accuminata* (leaves),

and *Musa accuminata* (trunk) respectively. The presence of phosphate solubilizing bacteria (PSB) and nitrogen solubilizing bacteria (NSB) throughout the decomposition period was confirmed by their growth on NBRIP and a modified nutrient medium that was specifically designed for the identification of ammonifiers respectively. Prepared fertilizer samples were applied to young tea plants that were grown in the Mawanella area in Sri Lanka (7˚15'12.42"N 80˚26'47.62"E) and according to the results, it is clear that fertilizer mixture 1 (N:P:K, 10:5:10, tea dust + *Gliricidia* + banana trunk) and fertilizer mixture 2 (N:P:K, 10:5:10, tea dust + *Gliricidia* + banana leaves) has the potential to increase the growth of young tea plants.

Keywords

Camellia sinensis, *Musa accuminata*, *Gliricidia sepium*, Phosphate Solubilizing Bacteria, Nitrogen Solubilizing Bacteria

1. Introduction

Plants require light, water, and some elements to support their biochemical needs. Nitrogen, phosphorous, potassium, magnesium, calcium, and sulfur are the micronutrients required for plants' growth. Nutrients that are required in trace amounts are manganese, iron, copper, zinc, boron, and molybdenum [1]. These elements naturally exist in soil; however, this may not be sufficient to sustain the agricultural lands. As a result, in modern farming, farmers use mineral supplements to their crops to improve productivity. Many farmers add supplements daily to extend the crop yield. The fertilizers used in agriculture contain essential nutrients, together with main elements such as nitrogen, potassium, and phosphorus that fall under either organic or inorganic fertilizers. These enhance the water retention capacity of soil while increasing soil fertility [2]. The main functions of inorganic/chemical fertilizers are briefed below.

Nitrogen fertilizers improve the plant's productivity and quality of agricultural products as it is the main constituent of chlorophyll that maintains the balance in the process of photosynthesis [3]. Nitrogen is also essential in the synthesis of amino acids, which is the building block of enzymes and proteins [3]. The nitrogen in fertilizers converts nitrogen to the plant-absorbable form of ammonium and nitrate ions by the soil microorganisms.

Nitrates are soluble in water and hence they will move with the soil water. During rainy seasons, the nitrates wash away and enter the drainage channels. This leaching process can be seen more often in rough-textured sandy soil. During the dry season, water evaporation causes nitrates to move upwards and accumulate at the soil surface. But if the nitrates leach below the root zone, it hinders the upward movement of nitrates. If the soil is saturated with water, soil organisms convert nitrates to gaseous nitrogen to acquire oxygen requirements for their metabolism. This process is called denitrification [4]. Ammonia is a gas

at atmospheric pressure, and it can be compressed into a liquid and can be applied to agricultural lands as anhydrous ammonia. Phosphorous is a major component in nucleic acids and plays an important part in cell growth and proliferation [2] and in complex energy transformations in plants [5].

There are two main types of potassium fertilizers in which potassium is combined with either sulfate (sulfate of potash) or chloride (muriate of potash) [6]. Potassium plays a major role in enzyme activation, movement of nutrients and water, stomata opening and closing, increase disease resistance of plants, maintaining turgor pressure, etc. [7].

1.1. Organic Fertilizers

Organic fertilizers are natural fertilizers attained from plants and animal debris. It upgrades the quality of the soil with carbonic compounds essential for plant growth. Organic fertilizers enrich the soil with organic matter, promote the growth and reproduction of microorganisms, and change the chemical and physical properties of the soil. It's considered to be one of the main nutrients for organic food. Organic fertilizers can be obtained from agricultural waste, livestock manure, municipal waste, and sludge [2]. Organic fertilizers increase the water-holding capacity, microbial growth, and soil structure [8] [9]. The drawbacks of organic fertilizers are the unequal distribution of nutrients, slow release of nutrients, inconsistent product composition, and requirement in high quantities [8] [9]. Besides, the world is slowly moving towards organic fertilizers to overcome the environmental problems caused by chemical fertilizers such as soil acidification, soil pH change, the release of greenhouse gases, the release of heavy metals, and the destruction of beneficial species, which ultimately cause severe impact on human health [10] [11]. Considering the drawbacks encountered in using the existing fertilizers, this project aims to produce organic fertilizers from readily available biomass that can be especially applicable to young tea plants.

1.2. Organic Materials That Can Be Used in Organic Fertilizers

Bananas originated in southwest Asia. Several modern varieties are now grown worldwide for human consumption. Ambul (*Musa acuminata Cavendish Subgroup*), Seeni (*Musa acuminata* "*Lady Finger*"), Kolikuttu (*Musa acuminata* × *M. balbisiana* (*AAB Group*) "Silk"), Ambun (*Musa acuminata* (AAA Group) "Gros Michel") and Anamalu (*Musa* "*Anamalu*") are the most common species that are grown in Sri Lanka. These varieties were formed by the cross between ordinary wild species *Musa accuminata* and *Musa balbesiana*. Banana is one of the most consumed fruits in Sri Lanka and it is also a very popular perennial crop among farmers. Due to its high demand, it is grown in approximately over 60,000 ha in Sri Lanka which is 54% of total fruit lands [12]. The average yield is 13 mt/ha per year yielding 780,000 mt of banana in Sri Lanka [13]. The banana tree is cut down after fruiting to facilitate new growth. During this process, tons of banana stems and leaves get wasted. Hence, this waste material can be used as

a raw material in the production of the proposed organic fertilizers.

Tea was first introduced to Sri Lanka in 1867 and it is known worldwide as Ceylon Tea. The highlands of Sri Lanka provide a favorable climate (humidity, rainfall, and cold temperatures) for high-quality tea production. Tea is also grown in low-altitude areas such as Galle, Matara, and Ratnapura districts in Sri Lanka. Ceylon Tea is one of the top agricultural exports in Sri Lanka and it is grown in 203,000 ha which is around 4% of the total land area in Sri Lanka. The total tea production in Sri Lanka is about 340 million kilograms per year. Tea must be dried, crushed, and processed before reaching the market. During this process, massive amounts of tea dust are produced which has a very low market value, and large amounts of tea dust are discarded every day. Discarded tea dust can be used in the manufacturing of supplements for pigs and it is also used in the preparation of activated tea waste charcoal that can be used in the adsorption field. Tea dust is also used in the production of cosmetics, fertilizers, and biofuels [14].

Therefore, there is a potential for using these discarded tea dust as organic fertilizers [15]. Hence, the second major ingredient selected for organic fertilizer is discarded tea dust.

Gliricidia sepium is native to Central America and Mexico. It is also found in Sri Lanka, India, Myanmar, Central Africa, and Southeast Asia. *Gliricidia sepium* is widely used as an intercropping plant and as living fences in Sri Lanka. This plant can grow in low fertile soils and it is well known for its nitrogen-fixing ability. *Gliricidia sepium* is one of the widely spread plants in Sri Lanka and it is not used for human consumption. Because of its high availability in different regions, the N, P, and K content of *Gliricidia sepium* leaves were analyzed to use them in organic fertilizers [16].

1.3. Microbial Analysis

Composting is the natural process of decomposing and recycling organic matter into a humus-rich soil amendment known as compost [17]. Organic matter found in food waste can be composted to produce fertilizer. Food waste can be easily disposed of in this environmentally friendly manner. By utilizing leftovers and other food waste, it can be used to create a highly organic product rich in nutrients for crop cultivation such as carbon-rich materials (waste papers, twigs, and leaves, and nitrogen-rich ones (grass, coffee, and tea grounds, fruit, and vegetables). The most important factors that affect the composting process are the pH, moisture content, temperature, C/N ratio, particle size, bulking agents, aeration, compaction, and nutrient balance [18]. Microorganisms need ideal moisture content because they act within the thin water film present on particles. During the composting process, microorganisms need sufficient amounts of nutrients to satisfy their energy requirements [18]. Temperature and pH are the other two important factors that affect the composting process. The optimum pH for composting is 5.5 - 8 and the optimum temperature is reported as

32˚C - 60˚C [19] [20]. If microbial growth is hindered due to low pH it will affect the temperature rise, and eventually decomposition process will slow down. All these factors are interrelated, therefore if microbial growth is affected by one factor it will result in changes in other factors as well.

During the decomposition process, plant material undergoes mechanical, chemical, and/or enzymatic reactions. Plant materials typically undergo eco-friendly retting processes that use complex microbial communities to accelerate the release of soluble components such as sugars, glycosides, and nitrogenous compounds [21]. Further growth of microbes is also facilitated by the release of simple monomers [22]. To confirm that scenario, the presence of microorganisms that are capable of phosphorus solubilization and nitrogen fixation was determined in each sample over the given period in addition to determining the change in these microbial loads in the sample.

Nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) are widely used to produce organic fertilizers. Microorganisms convert indigestible inorganic compounds into digestible form by converting organic matter in fertilizer into mineral forms [23]. To evaluate the contribution of these microbes in the decomposition process, the growth pattern of microbes within all four sample sets (*Camelia sinesis*—Tea dust, *Gliricidia sepium*—leaves, *Musa acuminata*—Banana trunk, and Banana leaves) was determined by examining the changing pattern of total plate count, and the presence of phosphorous solubilizing bacteria and nitrogen solubilizing bacteria, which are capable of ammonification (AMM) within the three-week study period.

2. Methodology

Ammonium oxalate (99%, Sigma-Aldrich),ammonium hydroxide (28% - 30%, Fisher Scientific), potassium dihydrogen phosphate (99.95%, Sigma-Aldrich), perchloric acid (99.99%, Thermo Scientific), sodium hydroxide (99.9%, DCM), ammonium molybdate (99.98%, Sigma-Aldrich), stannous chloride (98%, Loba Chemie), H_2SO_4 (98%, Sigma-Aldrich), $Na_2S_2O_3$ (99.9%, Sigma-Aldrich), boric acid (99.5%, Sigma-Aldrich), HCl (37%, Fisher Scientific, Agar Sigma-Aldrich).

2.1. Analysis of Potassium

2.1.1. Sample Preparation

Accurately weighed out 2.5 g of the sample (*Camellia sinensis*, *Gliricidia sepium*, *Musa accuminata* (leaves), and *Musa accuminata* (trunk)) (previously grounded and sieved with a B.S.S. No.60) was transferred to a 400 ml beaker of deionized water (125 ml) and of saturated ammonium oxalate (50 ml) were then added. Next, it was gently boiled for 30 minutes. The solution was cooled, and an ammonium hydroxide solution (5.0 M, 55 ml) was added. The solution was allowed to cool and transferred to a 250 ml volumetric flask and diluted to the volume. Then the solution was mixed and filtered a portion through a dry Whatman No.

30 filter paper into a clean, dry 250 ml beaker. Then the 25.00 ml aliquot of the filtrate was transferred to a 500 ml volumetric flask and diluted to volume with deionized water and mixed well. A suitable aliquot was transferred to a 100 ml volumetric flask so that the final solution contained approximately 16 ppm K_2O. Finally, the sample was diluted to 100 ml with deionized water and mixed well [24].

2.1.2. Standard Preparation

Accurately weighed 5.779 g of potassium dihydrogen phosphate (previously dried for one hour at 105˚C) was dissolved in deionized water (50 ml) and transferred to a 1000 ml volumetric flask and diluted to volume with deionized water to prepare a 2000 ppm K_2O solution. A 50 ml portion of it was transferred to a 1000 mL volumetric flask and diluted with deionized water to obtain a 100 ppm solution of K_2O. Further standards were prepared containing 10, 12, 14, 15, 16, 17, 18 and 20 ppm K_2O [24].

2.1.3. Method

A calibration curve for potassium was plotted by running the above samples in the Jenway flame photometer, the unknown sample was aspirated into the instrument following the given operating procedures, and a calibration curve was used to calculate the unknown potassium concentration [24].

2.2. Analysis of Phosphorous

The stannous chloride method of phosphorous analysis described in [25], with slight modifications, was followed to determine the total phosphate content in each of the samples.

Total Phosphate Content

Weighed out 3.0 g of sample (air-dried and finely ground) into a reagent bottle and the extracting solution (100.0 ml) was added, stoppered, and shook for about 30 minutes. Allowed to stand for 15 minutes and filtered through a folded filter paper. A 25.00 ml of the extract solution was pipetted out into a flask and evaporated to dryness. Cooled and dissolved the residue in 1 ml of perchloric acid. The flask was gently heated so that the content became colorless. The solution was allowed to cool and distilled water (10 ml) and phenolphthalein indicator (2 drops) were added. Titrated the solution with sodium hydroxide solution (6.0 M) until the color changed to pink. Made up the volume of the titrated solution to 25 ml with distilled water. Next, ammonium molybdate solution (0.06 M, 1 ml) and stannous chloride solution (0.08 M, 3 drops) were added. Kept the solution for 5 minutes and recorded the absorbance on a spectrophotometer at 690 nm. Measured the absorbance of distilled water blank in a similar manner. Different concentrations of standard phosphorous solution were treated in a similar manner and plotted a standard curve between absorbance and concentration of standard phosphorous solutions.

2.3. Analysis of Nitrogen

The nitrogen content was determined using the micro-Kjeldahl method with slight modifications. Exactly 1.00 g/1.00ml of sample was added to a digesting tube into which conc. H_2SO_4 (25.00 ml) and one catalyst tablet (kjletabs Cu-3.5, Foss TM) were added. Tubes were heated to a high temperature (350˚C, 30 min) for digestion. The digested samples were diluted with distilled water (100.0 ml) and NaOH (40%, 10.0 ml). Thereafter, $Na_2S_2O_3$ (5.0 ml) was introduced as an anti-bumping agent, and then the sample was distilled with boric acid (4%, 10.0 ml). The NH3 content in the distillate was determined by titrating with HCl (0.1 M). Also, a blank was prepared without the sample [26].

2.4. Decomposition of Sample in Water

100.00 g of each sample (*Camellia sinensis*, *Gliricidia sepium*, *Musa accuminata* (leaves), and *Musa accuminata* (trunk)) was crushed and dissolved in 100.00 ml of water and kept for decomposition at room temperature. 1.00 ml of each sample was taken at different time intervals (7, 14, and 21 days) to analyze dissolved nitrogen, potassium, and phosphorus.

2.5. Application of Fertilizer Samples

Prepared fertilizer samples (fertilizer mixture 1; N:P:K, 10:5:10, tea dust + *Gliricidia* + Banana trunk, fertilizer mixture 2; N:P:K, 10:5:10, tea dust + *Gliricidia* + banana leaves, fertilizer mixture 3; 10:5:15, tea dust + *Gliricidia* + banana trunk decomposed solution)were applied to tea plants (60 day's old) that were grown in Mawanella area (7˚15'12.42"N 80˚26'47.62"E). This test was carried out from February to May (2022) which provides the ideal weather conditions for the tea plantation. All of these organic fertilizer samples were prepared at the research facility recreating natural conditions on a small scale and the farmer was instructed by the research team on the fertilizer application process (note: no technicians were involved).

2.6. Statistical Analysis

Data obtained in triplicates were processed by the one-way analysis of variance (ANOVA) technique. Tukey's multiple comparison test was used to identify the means that differ significantly at $p < 0.05$. Results were expressed as mean ± standard deviation, the standard deviation of triplicate for nitrogen, phosphorous, and potassium content.

2.7. Enumeration of Microorganisms

The presence of bacteria in each sample over the decomposition period was quantified by using the dilution plate method. For the preparation of 10^{-1} dilution of each sample, 1.0 g of each solid sample was added into a separate stomacher bag with 9.0 mL of peptone. Then the sample was stomached for the preparation of a homogenized sample which represents 10^{-1} dilution. Then the

rest of the dilutions were prepared by using the same diluent until the concentration reached 10^{-8}. 0.1 ml of each diluted sample was added aseptically to the plate containing Plate Count Agar (PCA) in duplicates. After spreading the sample on the surface of the PCA medium under aseptic conditions, plates were incubated at 37˚C for 48 hours. After the completion of the incubation period, colony counts were measured in each plate containing not more than 300 but greater than 30. Colonies of two consecutive dilutions were counted and were calculated according to the following formula.

$$N = \sum C / \left(n_1 + 0.1 n_2 \right) d$$

where,

N = No of microorganisms/ml or/gram of product.

$\sum C$ = Sum of colonies on all dishes considered.

d = dilution corresponding to 1st dilution.

n_1 = Number of plates retained in the 1st dilution.

n_2 = Number of plates retained in the 2nd dilution.

2.8. Preparation of Pre-Enrichment Samples

Before culturing microorganisms on selective media plates, the pre-enrichment step was done. For that, a representative colony amount of each sample was taken from the plate of 10^{-6} dilution, which was the best dilution that produced well-isolated bacterial colonies for all four samples and inoculated into individual tubes containing 10.0 ml of sterile nutrient broth. All inoculated nutrient broth tubes were incubated at 37˚C for 48 hours.

2.9. Isolation of Nitrogen-Fixing Bacteria (NFB)

According to the method of [27] with slight modifications, the presence of ammonifying bacteria in each sample over the given period was confirmed by using a nutrient medium (g/l) [peptone 10.0; NaCl 5.0; ammonium ferric citrate 0.30; agar 15.0; yeast extract 2.0; sterilized water (pH 7.0)] that usually used to identify the presence of ammonifiers present. Then, the in-depth method was used, introducing 1.0 ml of each diluted sample into the melted and cooled nutrient medium in Petri dishes. Petri dishes were incubated in a thermostat at 28˚C in the dark for 48 hours. If there are bacteria in the sample that fix nitrogen, black colonies should form as a result of the release of H_2S.

2.10. Isolation of Phosphate Solubilizing Bacteria (PBS)

According to the method of [23] with slight modifications, the presence of PSB was confirmed. Herein, 0.1 ml of each diluted sample was spread directly onto the surface of NBRIP culture medium plates and incubated at 35˚C ± 2˚C for seven days. At the end of the incubation period, the presence of PSB was confirmed by observing the presence of a clear zone around bacterial colonies of each sample.

2.11. Decision on the Type of Fertilizer Mixture and Dose

If the available soil potash is below 100 ppm, prepare a 10:5:15 fertilizer mixture (N:P:K by w/w) for application and if the available soil potash is above 100 ppm, prepare a 10:5:10 fertilizer mixture for application. If the N:P:K ratio is 10:5:10 one tea plant requires 6.0 g of nitrogen, 3.0 g of phosphorous, and 6.0 g of potassium for 6 - 8 weeks. If the N:P:K ratio is 10:5:15 one tea plant requires 6.0 g of nitrogen, 3.0 g of phosphorous, and 9.0 g of potassium for 6 - 8 weeks [27].

3. Results and Discussion

Table 1. Results of nitrogen, phosphorous, and potassium analysis in raw samples.

Sample	mg/100g of sample		
	Nitrogen	Phosphorous	Potassium
Camellia sinensis (Tea Dust)	116.80 ± 0.08	66.00 ± 0.14	25.40 ± 0.01
Gliricidia sepium (Leaves)	43.60 ± 0.03	27.67 ± 0.04	22.40 ± 0.03
Musa acuminata (Banana Trunk)	13.00 ± 0.70	21.67 ± 0.01	106.80 ± 0.04
Musa acuminata (Banana Leaves)	10.54 ± 0.04	20.16 ± 0.03	104.50 ± 0.03

The values are mean ± standard deviation of the replicates.

Table 2. Results of nitrogen content in 100.00 ml of water after 3 weeks of decomposition.

	mg/100.00ml			
Decomposition time (weeks)	*Camellia sinensis* (Tea dust)	*Gliricidia sepium* (Leaves)	*Musa acuminata* (Banana leaves)	*Musa acuminata* (Banana trunk)
0	$6.80^{A} \pm 0.85$	$4.60^{A} \pm 1.13$	$1.67^{A} \pm 0.01$	$1.80^{A} \pm 0.57$
1	$9.47^{B} \pm 0.04$	$7.88^{B} \pm 0.11$	$1.85^{B} \pm 0.01$	$2.20^{A} \pm 0.70$
2	$13.46^{C} \pm 0.07$	$14.44^{C} \pm 0.05$	$2.13^{C} \pm 0.02$	$2.53^{A} \pm 0.57$
3	$16.90^{D} \pm 0.42$	$21.50^{D} \pm 0.11$	$2.42^{D} \pm 0.04$	$2.60^{A} \pm 0.11$

The values are mean ± standard deviation of the replicates.

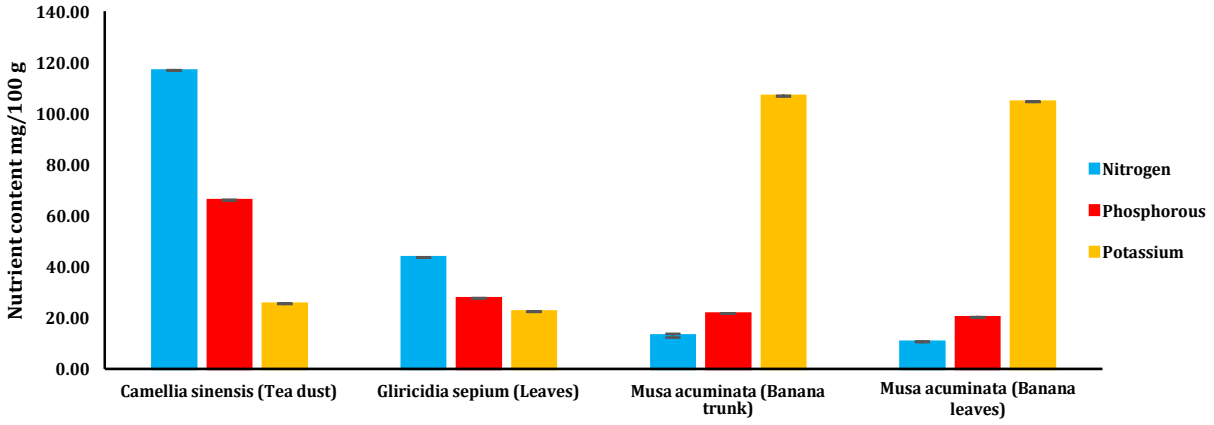

Figure 1. Nutrient content of *Camellia sinensis*, *Gliricidia sepium*, and *Musa acuminata* (Trunk and Leaves) in mg in 100 g of the sample. Bars represent the mean ± standard deviation.

The values with common superscript letters in each column are not significantly different ($p < 0.05$).

Table 3. Results of potassium content in 100.00 ml of water after 3 weeks of decomposition.

	mg/100.00ml			
Decomposition time (weeks)	*Camellia sinensis* (Tea dust)	*Gliricidia sepium* (Leaves)	*Musa acuminata* (Banana leaves)	*Musa acuminata* (Banana trunk)
0	$8.04^{A} \pm 0.23$	$6.80^{A} \pm 0.85$	$34.18^{A} \pm 0.06$	$34.80^{A} \pm 0.85$
1	$8.10^{A} \pm 0.03$	$6.94^{A} \pm 0.08$	$36.04^{B} \pm 0.72$	$36.54^{A} \pm 0.07$
2	$8.96^{B} \pm 0.06$	$7.90^{A} \pm 0.42$	$39.86^{C} \pm 0.03$	$45.61^{B} \pm 0.07$
3	$9.80^{C} \pm 0.08$	$8.74^{A} \pm 0.13$	$43.21^{D} \pm 0.01$	$47.00^{B} \pm 0.42$

The values are mean ± standard deviation of the replicates.

The values with common superscript letters in each column are not significantly different ($p < 0.05$).

Table 4. Results of phosphorous content in 100.00 ml of water after 3 weeks of decomposition.

	mg/100.00ml			
Decomposition time (weeks)	*Camellia sinensis* (Tea Dust)	*Gliricidia sepium* (Leaves)	*Musa acuminata* (Banana Leaves)	*Musa acuminata* (Banana Trunk)
0	$4.05^{A} \pm 0.21$	$2.67^{A} \pm 0.10$	$4.34^{A} \pm 0.03$	$5.00^{A} \pm 0.30$
1	$5.39^{B} \pm 0.07$	$2.86^{A} \pm 0.07$	$4.88^{B} \pm 0.03$	$6.55^{B} \pm 0.08$
2	$7.22^{C} \pm 0.04$	$3.82^{B} \pm 0.08$	$5.28^{C} \pm 0.04$	$7.28^{C} \pm 0.04$
3	$8.65^{D} \pm 0.06$	$4.63^{C} \pm 0.08$	$5.76^{D} \pm 0.01$	$7.47^{C} \pm 0.15$

The values are mean ±standard deviation of the replicates.

The values with common superscript letters in each column are not significantly different ($p < 0.05$).

Table 5. Fertilizer mixture 1 for 10:5:10 ratios (Tea dust + *Gliricidia* + Banana trunk).

Sample/100g	N	P	K
Camellia sinensis (Tea Dust) 100.00 g	4.60	2.60	1.00
Gliricidia sepium dissolved water (after 3 weeks of decomposition) 100.00 ml	4.64	1.00	1.89
Musa acuminata (Banana Trunk) 100.00 g	1.00	1.67	8.22
Total	10.24	5.27	11.11

Table 6. Fertilizer mixture 2 for 10:5:10 ratios (Tea dust + *Gliricidia* + Banana leaves).

Sample	N	P	K
Camellia sinensis (Tea Dust) 100.00 g	4.60	2.60	1.00

Continued

Gliricidia sepium dissolved water (after 3 weeks of decomposition) 100.00 ml	4.64	1.00	1.89
Musa acuminata (Banana Leaves) 100.00 g	1.00	1.91	9.91
Total	10.24	5.51	12.8

Table 7. Fertilizer mixture 3 for 10:5:15 ratios (Tea dust + *Gliricidia* + Banana trunk decomposed solution).

Sample	N	P	K
Camellia sinensis (Tea Dust) 100.00 g	4.60	2.60	1.00
Gliricidia sepium dissolved water (after 3 weeks of decomposition) 100.00 ml	4.64	1.00	1.89
Musa acuminata dissolved in water after 1 week of decomposition (Banana Trunk) 100.00 ml	1.00	2.98	16.61
Total	10.24	6.58	19.50

Table 8. The table of results for the total plate count.

Decomposition time (Weeks)	Microbial count in each sample (CFU/mL)			
	Camellia sinensis (Tea dust)	*Gliricidia sepium* (Leaves)	*Musa acuminata* (Banana leaves)	*Musa acuminata* (Banana trunk)
0	2.51×10^3	9.6×10^5	7.75×10^5	1.89×10^6
1	1.78×10^5	1.7×10^6	1.7×10^6	4.22×10^6
2	8.71×10^5	5.49×10^8	5.49×10^7	1.61×10^7
3	3.28×10^6	1.21×10^{10}	2.18×10^8	6.49×10^7

Table 9. The effect of fertilizer mixture 1 on young tea plants.

Plant	Plant height/cm			Number of leaves			Length of the leaf/cm			Width of the leaf/cm		
Duration	60 days	90 days	120 days	60 days	90 days	120 days	60 days	90 days	120 days	60 days	90 days	120 days
P0 (Control)	$18.00^A \pm 0.36$	$22.00^B \pm 0.36$	$24.80^C \pm 0.36$	$6^A \pm 1.00$	$9^B \pm 1.00$	$15^C \pm 1.00$	$4.00^A \pm 0.12$	$5.30^B \pm 0.12$	$6.20^C \pm 0.12$	$1.90^A \pm 0.10$	$2.50^B \pm 0.10$	$2.80^B \pm 0.10$
P1	18.30	25.40	30.80	5	10	18	4.30	5.60	6.40	2.00	2.50	3.30
P2	17.50	25.20	30.10	6	10	16	3.70	5.50	6.20	1.80	2.50	3.00
P3	18.80	25.70	31.80	8	16	22	4.00	5.90	6.60	1.90	2.70	3.00
P4	18.20	25.40	30.50	7	13	18	4.10	5.50	6.50	2.00	2.50	3.10
P5	18.00	25.90	30.30	7	11	18	3.90	5.30	6.30	1.90	2.40	3.00
Mean	$18.16^A \pm 0.32$	$25.52^B \pm 0.32$	$30.70^C \pm 0.32$	$6.60^A \pm 1.30$	$12.00^B \pm 1.30$	$18.40^C \pm 1.30$	$4.00^A \pm 0.13$	$5.56^B \pm 0.13$	$6.40^C \pm 0.13$	$1.92^A \pm 0.07$	$2.52^B \pm 0.07$	$3.08^C \pm 0.07$
Sig. (2-tailed) between P0 and Mean	0.499	0.000	0.000	0.273	0.129	0.051	0.780	0.091	0.069	0.350	0.453	0.019

The values are mean ± standard deviation of the replicates.

The values with common superscript letters in each column are not significantly different ($p < 0.05$). (P1, P2, P3, P4 and P5 are the plants that were fertilized)

Table 10. The effect of fertilizer mixture 2 on young tea plants.

Plant	Plant height/cm			Number of leaves			Length of the leaf/cm			Width of the leaf/cm		
Duration	60 days	90 days	120 days	60 days	90 days	120 days	60 days	90 days	120 days	60 days	90 days	120 days
P0 (Control)	$17.50^{A} \pm 0.22$	$21.30^{B} \pm 0.22$	$23.20^{C} \pm 0.22$	$6^{A} \pm 0.41$	$10^{B} \pm 0.41$	$15^{C} \pm 0.41$	$4.30^{A} \pm 0.70$	$5.20^{B} \pm 0.70$	$6.00^{C} \pm 0.70$	$2.00^{A} \pm 0.60$	$2.40^{B} \pm 0.60$	$2.70^{C} \pm 0.60$
P1	17.00	23.00	28.60	6	10	17	4.10	5.10	6.10	2.00	2.40	2.80
P2	18.00	23.80	29.00	6	11	18	4.70	5.60	6.40	2.10	2.50	3.10
P3	18.00	24.70	30.20	7	12	18	4.40	5.30	6.30	2.00	2.50	3.20
P4	18.00	25.10	29.50	6	12	17	4.80	5.70	6.40	2.00	2.40	2.90
P5	17.00	24.00	29.10	7	11	20	4.00	5.20	6.20	1.80	2.40	2.80
Mean	$17.60^{A} \pm 0.42$	$24.12^{B} \pm 0.42$	$29.28^{C} \pm 0.42$	$6.40^{A} \pm 0.58$	$11.20^{B} \pm 0.58$	$18.00^{C} \pm 0.58$	$4.40^{A} \pm 0.17$	$5.38^{B} \pm 0.17$	$6.28^{C} \pm 0.17$	$1.98^{A} \pm 0.08$	$2.44^{B} \pm 0.08$	$2.96^{C} \pm 0.08$
Sig. (2-tailed) between P0 and Mean	1.000	0.009	0.000	0.374	0.113	0.014	0.597	0.292	0.022	0.742	0.374	0.075

The values are mean ± standard deviation of the replicates.

The values with common superscript letters in each column are not significantly different ($p < 0.05$). (P1, P2, P3, P4 and P5 are the plants that were fertilized)

Table 11. The effect of fertilizer mixture 3 on young tea plants.

Plant	Plant height/cm			Number of leaves			Length of the leaf/cm			Width of the leaf/cm		
Duration	60 days	90 days	120 days	60 days	90 days	120 days	60 days	90 days	120 days	60 days	90 days	120 days
P0 (Control)	$18.00^{A} \pm 0.09$	$22.00^{B} \pm 0.09$	$25.00^{C} \pm 0.09$	$5^{A} \pm 0.40$	$9^{B} \pm 0.40$	$13^{C} \pm 0.40$	$4.00^{A} \pm 0.06$	$5.00^{B} \pm 0.06$	$5.90^{C} \pm 0.06$	$2.10^{A} \pm 0.06$	$2.40^{B} \pm 0.06$	$2.80^{C} \pm 0.06$
P1	18.90	24.10	29.00	7	10	18	4.00	5.50	6.40	2.00	2.60	3.00
P2	18.40	24.00	29.00	5	10	17	4.60	6.00	7.00	2.20	2.70	3.00
P3	18.30	24.30	30.00	6	12	19	4.00	5.40	6.50	2.00	2.70	3.10
P4	18.50	20.00	-	3	3	-	3.00	3.20	-	1.50	1.60	-
P5	18.00	23.00	29.00	7	10	18	4.50	5.60	6.60	2.10	2.70	3.30
Mean	$18.42^{A} \pm 0.71$	$23.08^{B} \pm 0.71$	$29.25^{C} \pm 0.76$	$5.60^{A} \pm 1.49$	$9.00^{A} \pm 1.49$	$18.00^{B} \pm 1.58$	$4.02^{A} \pm 0.49$	$5.14^{A} \pm 0.49$	$6.63^{B} \pm 0.52$	$1.96^{A} \pm 0.21$	$2.46^{A} \pm 0.21$	$3.10^{B} \pm 0.23$
Sig.(2-tailed) between P0 and Mean	0.147	0.498	0.000	0.652	1.000	0.003	0.968	0.918	0.018	0.677	0.875	0.034

The values are mean ± standard deviation of the replicates.

The values with common superscript letters in each column are not significantly different ($p < 0.05$). (P1, P2, P3, P4 and P5 are the plants that were fertilized)

Table 12. Physical parameters that were recorded during the fertilizer application process from March to May 2022.

Parameters	February	March	April	May
Max Temp/˚C	30	32	31	30
Min Temp/˚C	19	20	21	22
Avg rainfall/mm	138.56	186.07	357.38	338.08
Humidity	77%	74%	79%	80%

[28].

4. Discussion

The nitrogen content of tea dust, *Gliricidia sepium*, and banana trunk was analyzed using the Kjeldahl method. Herein, the sample was digested with concentrated sulphuric acid and then the ammonium ions were converted to ammonia in the distillation stage. This ammonia was captured by boric acid, and it was titrated with an acid.

$$\text{Sample}_{(s)} + H_2SO_{4(aq)} \rightarrow (NH_4)_2\,SO_{4(aq)} + CO_{2(g)} + SO_{2(g)} + H_2O_{(l)}$$

$$(NH_4)_2\,SO_{4(aq)} + 2NaOH_{(aq)} \rightarrow 2NH_{3(g)} + Na_2SO_{4(aq)} + H_2O_{(l)}$$

$$NH_{3(g)} + H_3BO_{3(aq)} \rightarrow NH_4^+H_2BO_{3(aq)}^-$$

$$NH_4^+H_2BO_{3(aq)}^- + H_2SO_{4(aq)} \rightarrow (NH_4)_2\,SO_{4(aq)} + 2H_3BO_{3(aq)}$$

Gliricidia sepium was traditionally popular as an organic fertilizer among small-scale farmers as it is known to be rich in nitrogen. In this study, it is revealed that tea dust contains 0.07% more nitrogen than *Gliricidia sepium* tested. The lowest nitrogen content was observed in banana leaves which is 10.54 ± 0.04 mg (**Table 1**). The percentage of nitrogen released into the water was measured by using the procedure given in section 2.3. This study reveals (**Table 2**) that nitrogen extraction into the water increased ($P < 0.05$) with decomposition time for tea dust, *Gliricidia sepium*, and banana Leaves. However, there is no significant change ($P > 0.05$) in the extraction of nitrogen by banana trunks. Though the highest percentage of nitrogen was found in tea dust (6.80^A ± 0.85 mg), after 3 weeks of decomposition *Gliricidia sepium* released more nitrogen into water than tea dust. During the decomposition process, no other chemicals were used to extract nitrogen as this study aimed at introducing a homemade organic fertilizer for small-scale farmers.

The potassium content was observed to be higher in the raw banana trunk (106.80 ± 0.04 mg) than in raw banana leaves (104.50 ± 0.030). Tea dust and *Gliricidia sepium* contained 25.40 ± 0.01 mg and 22.40 ± 0.03 mg respectively (**Figure 1**). The potassium content released by all these samples was analyzed in

water by immersing them in water for 3 weeks. It was observed that the banana trunk and banana leaves are capable of leaching much of its potassium content to water over tea dust and *Gliricidia sepium*. According to the results, there was an overall increase ($P < 0.05$) in potassium concentration of tea dust, banana leaves, and banana trunks after three weeks of decomposition (Table 3). However, the potassium concentration of *Gliricidia sepium* has not changed significantly ($P > 0.05$) during the decomposition period. As the most cultivated fruit plant that creates a lot of waste biomass, the banana is a promising source of potassium for crops.

Plant intake phosphorous in the form of $H_2PO_4^-$ and HPO_4^-. The total phosphorous content of all four samples was measured by the Troug method, in this method, ammonium molybdate forms a blue-colored complex in the presence of a reducing agent. As shown in Figure 1, the highest phosphorous content was found in tea dust (66.00 ± 0.14 mg) and the lowest was recorded in banana leaves (20.16 ± 0.03 mg). When these samples were allowed to decompose in water, phosphorous release by tea dust, *Gliricidia sepium*, banana leaves, and banana trunk increased ($P < 0.05$) significantly (Table 4).

$$PO_{4(aq)}^{3-} + MoO_{4(aq)}^{2-} + 27H_{(aq)}^{+} \rightarrow H_3PO_4\left(MoO_3\right)_{12(aq)} + 2H_2O_{(l)}$$

$$H_3PMo\left(VI\right)_{12}O_{40(aq)} + \text{Reductant} \rightarrow \underbrace{\left[H_4PMo\left(VI\right)_8 Mo\left(V\right)_4 O_{40}\right]_{(aq)}^{3-}}_{\text{Blue Color Complex}}$$

The aim of this study is to prepare an organic fertilizer mixture for young tea plants using readily available biomasses. For young tea plants the required N:P:K ratio is 10:5:10 or 10:5:15 depending on the soil potash content. After careful analysis of the main nutrients in selected biomasses (both raw and decomposed), the required number of biomasses as fertilizers to fulfill the NPK requirement for tea plants was calculated. Different formulas were used to prepare the recommended N:P:K ratio. Tables 5-7 represent the closest N:P:K ratio to the recommended ratio mentioned above that can be obtained by using these materials. According to the results, it is clear that the ratios obtained in Table 5 and Table 6 are close to the 10:5:10 ratio. However, there is a difficulty in obtaining the 10:5:15 accurately as shown in Table 7. In mixture 3 potassium content is considerably higher than the recommended value.

The results in Total Plate Counts (Table 8), microbial growth has increased with the time in each sample over a three-week period as in previous studies [29] which analyzed the growth pattern of bacteria. Also, the results obtained for total plate count in tea dust, which is the sample with the highest solubilized N, and P contents depict the highest increment in microbial load with time, while the lowest microbial growth observed in *Musa acuminata* (Banana leaves), which has the lowest content of N and P.

According to the results obtained for the colony morphology on modified nutrient media plates, which were specifically designed to observe the growth of ammonifying bacteria, there were colonies staining the nutrient medium in black in all four samples due to the release of H_2S until the end of third week

with the increment of the number of colonies. It confirms the presence of ammonifying bacteria that are capable of degrading organic nitrogen (DON) to lower-weight molecular compounds, including free amino acids, amino sugars, urea, nucleic acids, uncharacterized labile compounds [30], and ultimately to ammonia, by a process termed ammonification. Meanwhile, the presence of phosphate-solubilizing bacteria was confirmed by the presence of microbial colonies with a clear zone on NBRIP media plates in all four samples. Due to having these types of colonies until the third week with an upward pattern, these results confirm the presence of nitrogen-solubilizing and phosphate-solubilizing bacteria in all four samples, and their growth has increased with the decomposition time, which can be used to confirm that the rate of solubilization of these ions as it has released monomer substances that accelerate the growth of those microorganisms in the sample.

According to the results in **Table 9**, in the control plant (P0) all the measured parameters have changed significantly except the width of the leaf from 90 days to 120 days. The mean values of measured parameters of test plants were calculated and significant differences were analyzed. It was observed that all the mean values of measured parameters have increased significantly ($p < 0.05$). The mean value is compared with the control plant (P0) and it was observed that plant heights are significantly different in unfertilized plants and fertilized plants. However, the number of leaves did not show any significant difference, but the numerical values show that there is a difference in the number of leaves between fertilized and unfertilized trees. The length and width of leaves did not change significantly during the application of the fertilizer except for the width of the leaf from 90 days to 120 days.

In mixture 2 control plant (P0) and mean values of test plants show a significant increase in their plant height, number of leaves, length of the leaf, and width of the leaf (**Table 10**). Same as in mixture 1 plant heights have changed significantly in fertilized tea plants but not the other parameters. The number of leaves and length of the leaf has increased significantly in the fertilized plants from 90 days to 120 days.

According to the results in **Table 11**, when mixture 3 was applied, it was observed that there was no significant difference in the number of leaves, length of the leaf, and width of the leaf in mean values of test plants, however, these parameters have significantly increased with time in the control plant (P0). When mean values were compared with the control plant it is clearly visible that most of the Sig. (2-tailed) values are higher than 0.05, therefore most of the parameters have remained the same in both fertilized and unfertilized plants. According to the statistical analysis of the test results, fertilizer mixtures 1 and 2 can be considered as potential organic fertilizers for young tea plants. Though mixture 3 has the recommended NPK ratio, this mixture must be further modified and tested to get feasible results. The efficiency of these mixtures in the actual field has not yet been tested therefore further studies have to be done in order to use

them in large-scale farms. All the fertilizer samples were applied from March to May, physical parameters that were observed during this period were given in Table 12. During this study influence of temperature on organic fertilizers was not studied, because during the experimental period minimum and maximum temperatures were nearly the same as shown in Table 12.

5. Conclusions

In this study, the nitrogen (N), phosphorus (P), and potassium (K) contents in raw and decomposed biomasses of *Camellia sinensis*, *Gliricidia sepium*, and *Musa acuminata* were investigated. The highest nitrogen and phosphorous content were observed in tea raw dust which was 116.80 ± 0.08 mg and 66.00 ± 0.14 mg. The highest potassium content was observed in the raw banana trunk, 106.80 ± 0.04 mg. When these samples were allowed to decompose in water, the nutrients (NPK) released to water were increased with time except potassium in *Gliricidia sepium* (leaves) and nitrogen in *Musa acuminata* (banana trunk). These samples were combined to form different fertilizer mixtures for young tea plants and field trials were conducted. Herein three fertilizer formulas were tested by mixing raw and decomposed biomass as reported in Tables 5-7. According to the growth parameters tested after applying the fertilizer formulas, the data were analyzed statistically which revealed that the fertilizer mixture 1 and 2 can be considered as potential organic fertilizers for young tea plants.

In the natural decomposition of organic wastes, bacteria can solubilize the phosphorus and nitrogen present in the organic materials, and their growth can also be accelerated by providing a growth-stimulating effect by simple monomers that are released by bacteria during the decomposition process. According to the results of the microbial growth on modified nutrient agar plates for the identification of ammonification bacteria and NBRIP media plates that were specifically used to identify phosphate solubilizing bacteria until the end of this natural decomposition process, it can be concluded that the contribution of nitrogen and phosphorous solubilizing bacteria is persisted over the concerned period. When fertilizer mixtures were applied to young tea plants, statistical analysis revealed that fertilizer mixture 1 and fertilizer mixture 2 increased the growth of young tea plants.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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