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Appendices

APPENDICES

APPENDIX – I

ESTIMATION OF LIGNIN (Goering and Van Soest, 1975)

Principle

Refluxing the sample material with acid detergent solution which removes the water soluble and materials other than the fibrous component. The left-out material is weighed after filtration, dried, treated with 72% H_2SO_4 and filtered, dried and ashed. The loss of weight on ignition gives the acid detergent lignin.

Reagents

Acid detergent solution (Dissolve 20 g of acetyl trimethyl ammonium bromide in one litre of 1 N sulphuric acid), 72% H_2SO_4 (W/V), acetone, round bottom flask and refluxing set, muffle furnace, sintered glass crucible – G2.

Procedure

A. Acid Detergent Fibre (ADF)

1g of powdered sample and 100ml of acid detergent solution was placed in a round bottom flask and boiled for 5 – 10 minutes. The heat was reduced to avoid foaming as boiling begins. Refluxing was done for 1 hour after the onset of boiling. Boiling was adjusted to slow, even level. The container was removed, swirled and filtered the contents through a preweighed sintered glass crucible (G2) by suction and washed with hot water twice. Then, washed with acetone and break up the lumps. Acetone washing was repeated until the filtrate was colorless. Dried at 100°C for overnight. Weighed after cooling in a desiccator. ADF content was expressed in percentage i.e., W/S x 100, Where W is the weight of the fibre and S is the weight of the sample.

B. Determination of Acid Detergent Lignin (ADL)

ADF was transferred to a 100 ml beaker with 25 - 50 ml of 72% sulphuric acid. 1g of asbestos was added to it. It was allowed to stand for 3 hours with an intermittent stirring with a glass rod. The acid was diluted with distilled water and filtered with preweighed Whatman No. 1 filter paper. The glass rod and the residue were washed several times to

get rid of the acid. The filter paper was dried at 100℃ and weighed after cooling in a desiccator. The filter paper was transferred to a preweighed silica crucible and ashed the filter paper with the content in a muffle furnace at 550℃ for about 3 h. The crucible was cooled in a desiccator and weighed. The ash content was calculated. 1 g asbestos was taken as blank and then added 72% H_2SO_4 and followed the steps from 2 - 5.

CALCULATION

$$\text{ADL (\%)} = \frac{\text{Weight 72\% } H_2SO_4 \text{ washed fibre (Test – Asbestos blank)} - \text{Ash (Test – Asbestos blank)}}{\text{Weight of sample}} \times 100$$

APPENDIX – II

ESTIMATION OF CELLULOSE (Updegroff, 1969)

Principle

Cellulose undergoes acetolysis with acetic/nitric reagent forming acetylated cello dextrin which get dissolved and hydrolyzed to form glucose molecules upon treatment with 67% H_2SO_4. This glucose molecule is dehydrated to form hydroxyl methyl furfural which forms green coloured product with anthrone and the colour intensity is measured at 630 nm.

Reagents

Acetic/Nitric reagent: 150 ml of 80% acetic acid was mixed with 15 ml of concentrated nitric acid. Anthrone reagent: 200 mg of anthrone was dissolved in 100 ml concentrated sulphuric acid and chilled for two hours before use. 67% sulphuric acid.

Procedure

A quantity of 0.1g of sample was taken in a test tube, to which 3 ml of acetic/nitric reagent was added and mixed well and kept in a water bath for 30 minutes. It was cooled and centrifuged for 15 - 20 minutes after which the supernatant was discarded. The residue was washed with distilled water and 10 ml of 67% sulphuric acid was added and allowed to stand for 1 hour. 1 ml of the solution was taken and diluted to 100 ml. From the above

diluted solution, 1 ml was taken to which 10 ml of anthrone reagent was added and kept in a boiling water bath for 10 minutes. It was then cooled and the absorbance was measured at 630 nm. A blank was set with anthrone reagent and distilled water. The amount of cellulose present in the sample was calculated using a standard graph corresponding to 40 - 200 µg of cellulose.

APPENDIX - III

ESTIMATION OF ORGANIC CARBON (Walkley and Black, 1934)

(Wet Chromic Acid Oxidation Method)

Principle

Organic carbon present in organic matter is oxidized by chromic acid in the presence of conc. H_2SO_4. Potassium dichromate on reaction of H_2SO_4 provides nascent oxygen which combines with carbon and form CO_2. The H_2SO_4 enables easy digestion of organic matter by rendering heat of dilution. Only a certain quantity of chromic acid is used for oxidation. The excess chromic acid left unused by the organic matter is determine by back titration with 0.5 N ferrous sulphate or ferrous ammonium sulphate using diphenylamine indicator.

Reagents

1 N potassium dichromate: Exactly 49.04 g of $K_2Cr_2O_7$ was dissolve in one litre of distilled water. Diphenylamine indicator: 0.5 g diphenylamine was dissolved in 20 ml of water and 100 ml of Conc. H_2SO_4 was added. 0.5 N ferrous sulphate or ferrous ammonium sulphate (139.0 g of ferrous sulphate or 196 g of ferrous ammonium sulphate was dissolved in 800 ml of distilled water. 20 ml of Conc. H_2SO_4 was added and the volume was made up to one litre). Conc. H_2SO_4, phosphoric acid (Orthophosphoric acid 85%).

Procedure

Exactly 0.5 g of soil (passed through 0.2 mm sieve) was weighed and transferred to 500 ml conical flask. 10 ml of 1N $K_2Cr_2O_7$ was added and mixed well by swirling the flask. Added 20 ml of conc. H_2SO_4 mixed by gentle rotation for one minute to ensure complete contact of the reagent with the soil. Allowed the contents to stand for

20-30 minutes. Kept the flask on asbestos sheet to avoid burning of table due to intense heat. Added 200 ml of water after 30 minutes. Then added 10 ml of phosphoric acid and 1 ml of diphenylamine indicator. Titrated the solution with 0.5 N ferrous ammonium sulphate. As the titration proceeds the dull green colour shifted to the turbid blue and at the end point bright green colour developed. Conducted simultaneously a blank titration (without soil) and the volume of 0.5 N ferrous ammonium sulphate consumed was noted.

CALCULATION

Weight of soil taken = 0.5 g

Volume of 1N $K_2Cr_2O_7$ = 10ml

Volume of 0.5N ferrous ammonium sulphate used for blank titration = X ml (Sample T. V)

Volume of 0.5N ferrous ammonium sulphate used for blank titration = Y ml (Sample T. V)

X ml of $FeSO_4$ reduces 10 ml of 1N $K_2Cr_2O_7$

Therefore Y ml of $FeSO_4$ reduces Y/X × 10 ml

Hence actual quantity of 1N $K_2Cr_2O_7$ used for oxidation of organic matter = 10 - (10 × Y/X)

1 ml of 1N $K_2Cr_2O_7$ = 0.003 g of 'C'

Therefore 10 – (10×Y/X) ml of 1N $K_2Cr_2O_7$ = 10 - (10 × Y/X) × 0.003

This is present in 0.5 g of soil

Therefore in 100 g = 10 - (10 × Y/X) × 0.003×100/0.5

Organic matter (surface soil) = organic carbon × 1.724

Organic matter (sub surface soil) = organic carbon × 2.5

APPENDIX - IV

ESTIMATION OF TOTAL NITROGEN (Humphries, 1956)

(Microkjeldhal Method)

Principle

A known weight of the powdered sample was treated with diacid mixture so as to oxidize the organic matter and bring the mineral elements into solution.

Reagents

Diacid mixture: 4:1 (w/w) ratio of concentrated sulphuric acid and concentrated perchloric acid. Mixed indicator: 0.5 g bromocresol green and 1 g of methyl red were dissolved in 100 ml of 90% ethyl alcohol. 40% sodium hydroxide solution. 2% boric acid. Concentrated sulphuric acid (0.02 N).

Procedure

A quantity of 0.2 g of dried, sieved and homogenized sample was taken in a micro kjeldhal digestion flask (50 ml capacity) to which 12 ml of diacid was added. Complete digestion was ensured by adding one drop of perchloric acid and the contents turns colourless like water. The volume was made upto 100 ml with distilled water. 10ml aliquot was pipette out into a Wagnor- Parnas distillation apparatus and 10ml of 2% boric acid with mixed indicator was kept in a beaker at the delivery end of the distillation apparatus. To the distillation apparatus, 10 ml of 40% sodium hydroxide was added and steam distilled. The distillate was collected until no more ammonia was evolved. The contents of the beaker were titrated against 0.02 N sulphuric acid until a red colour was appeared.

Total nitrogen content of the sample was determined by the formula.

$$\text{Total nitrogen (\%)} = \frac{0.00028 \times T.V \times 100 \times 100}{10 \times 0.2}$$

Where,

T.V	=	Titre value.
0.00028	=	1ml of 0.02 N sulphuric acid utilized.
10	=	Volume of extract taken for distillation (ml).

0.2	=	Weight of sample (g).
100	=	Total volume (ml).

APPENDIX - V

ESTIMATION OF TOTAL PHOSPHORUS (Jackson, 1973)

Principle

Phosphorus is precipitated as ammonium phosphomolybdate in nitric acid medium. The precipitate is filtered, washed free of acid, dissolved in a known excess of standard alkali and the excess alkali is determined by back titration with a standard acid using phenolphthalein indicator.

Reagent

Hydrochloric acid – 1:1, Nitric acid – 1:1, Conc. ammonium hydroxide, Conc. nitric acid, Solid ammonium nitrate, Ammonium molybdate solution – 20 percent, Potassium hydroxide – 0.1619N, Nitric acid - 0.1619N, Phenolphthalein.

Procedure

200 ml of HCl extract of the sample was pipette out into a 400 ml beaker and evaporated to a small bulk. Then, it was transferred to a silica basin using hot water and evaporated to dryness over a water bath. The silica basin was kept in an air oven at 105 to 110°C for 3 h to dehydrate the silica. This residue was dissolved by adding a small quantity of 1:1 hydrochloric acid and evaporated to dryness over a water bath. The residue was again dissolved in nitric acid adding sufficient amount of nitric acid to dissolve the same. The insoluble silica was allowed to settle overnight and then filtered through No.42 filter paper and the residue was washed in the silica basin and on the filter paper with small quantities of 1:4 nitric acid till no yellow colour was left either in the basin or in the filter paper. The filtrate was collected in a 250 ml beaker. The extract was made alkaline with conc. ammonium hydroxide. To this, 5 g of solid ammonium nitrate was added and kept on a thermostat at 65°C for 15 minutes. The precipitant mixture was prepared by taking 7 ml of conc. nitric acid and 3 ml of distilled water in a 100 ml beaker and 10 ml of 20 percent ammonium molybdate was added to this solution drop by drop with constant

stirring. 10 ml of this precipitant mixture was added drop by drop to the beaker in the thermostat with constant stirring and kept in the thermostat for another half an hour at 65°C and allowed the precipitate to settle well. Then, it was filtered through No.40 filter paper by decantation pouring only the supernatant liquid to the filter paper. The precipitate was then washed with cold distilled water till the filtrate runs free of acid. The filter paper was then transferred with the precipitate to the same beaker in which precipitation was done and enough water was added to make the filter paper into a pulp. Now, 0.1619N KOH was added from the burette, till the yellow precipitate was completely dissolved leaving a colourless solution. Then another 5 ml of 0.1619N KOH was added to keep the alkali in fair excess quantity. A drop of phenolphthalein was added and the excess alkali was titrated against 0.1619N nitric acid. Disappearance of pink colour indicated the end point.

CALCULATION

Weight of sample taken = W g

Volume of HCL extract prepared = 500 ml

Volume of HCL extract pipette out for analysis = 200 ml

Volume of 0.1619N KOH added in excess = a ml

Volume of 0.1619N HNO_3 used for back titration = b ml

Therefore, actual volume of 0.1619N KOH

used to dissolve the precipitate = (a-b)

1 ml of 0.1619N KOH = 0.0005gm P_2O_5

(a-b) ml of 0.1619N KOH = 0.0005 x (a-b) x gm P_2O_5

This was present in 200 ml of HCL extract

Therefore, in 500 ml = 0.0005 x (a-b) x 500/200

This was present in W gm of sample

Therefore, in 100 gm = 0.0005 x (a-b) x 500/200 x100/W

Percentage of P_2O_5 on moisture free basis = 0.0005 x (a-b) x 500/200 x 100/W x 100/(100 – M)

(M – Moisture content of the sample)

APPENDIX - VI

ESTIMATION OF TOTAL POTASSIUM (Jackson, 1973)

(Flame Photometer Method)

Principle

Certain elements when excited in flame emit radiation. The excitation causes one of the outer electrons of neutral atoms to jump to an outer orbit of higher energy level or the atoms may be excited sufficiently to lose an electron completely. When excited atoms return to lower energy levels light of characteristics wavelength is emitted. The flame photometer measures this radiation

intensity which is proportional to the concentration in a solution.

Preparation

1.907 g of KCL was dissolved in 1 litre of distilled water (1000 ppm of K). From this, various standards were prepared ranging from 10 to 100 ppm.

Procedure

The atomizer was fixed in its place and introduced with distilled water. The compressor was started and the air pressure was adjusted to 10 psi. The gas was opened to light the burner through the window. Flow of gas was adjusted to give a central bluish cone. Zero was set with distilled water by using the zero-adjustment knob. Then, 100 ppm K solution was introduced and adjusted to read 100 on the scale. Again distilled water was introduced and adjusted to zero. This process was repeated till without zero adjustment. Then various standard solutions were introduced the readings were recorded and the standard curve was drawn. The filtrate was taken from sesquioxide estimation in a small vial and introduced through the atomizer. The readings were recorded and the percentage of K was calculated by using the standard curve.

CALCULATION

Weight of sample taken = W g

Volume of HCL extract prepared = 500 ml

Volume HCL extract pipette out for

sesquioxide estimation = 50 ml

Volume of sesquioxide filtrate made up to = 250 ml

Metre reading = G

Equivalent ppm from standard curve = A

i.e. 1 ml of the solution contains

A microgram of K = A/106 g of K

Therefore, in 250 ml of the solution = A/106 x 250

This was present in 50 ml of HCL extract

Therefore, in 500 ml = A/106 x 250 x 500/50 g

This was present in W gm of sample

Therefore, in 100 gm = A/106 x 250 x 500/50 x 100/W g

Percentage of K on moisture free basis = A/106 x 250 x 500/50 x 100/W x 100/(100 – M)

(M – Moisture content of sample)

APPENDIX- VII

ESTIMATION OF CALCIUM AND MAGNESIUM (Jackson, 1973)

(Versanate Method)

Principle

Calcium and magnesium get complexed by EDTA in the order calcium first followed by magnesium. Calcium is estimated first by using murexide indicator at pH 12 in the presence of sodium hydroxide. Then calcium and magnesium is estimated using Erichrome Black – T at pH 10 in the presence of ammonium chloride and ammonium hydroxide buffer solution.

Reagents

0.02 N EDTA, 10% sodium hydroxide, Ammonium chloride – ammonium hydroxide buffer solution, Murexide solution, Erichrome Black – T indicator.

Procedure

Calcium alone

Pipette out 10 ml of seaqui oxide filtrate into a porcelain basin. Add 10% sodium hydroxide solution drop by drop to neutralize the activity (red litmus turns blue) and another 5 ml excess to maintain the pH at 12. Add a pinch (50 mg) of murexide indicator and titrate with 0.02N EDTA till the colour changes from pinkish red to purple or violet.

Calcium and Magnesium

Pipette out 10 ml of seaqui oxide filtrate into a porcelain basin. Add ammonium chloride – ammonium hydroxide buffer solution drop by drop to neutralize the acidity (use red litmus paper) and 5 ml excess to maintain the pH at 10. Add 2 – 3 drops of Erichrome Black – T indicator solution and titrate with 0.02 N EDTA till the colour changes from purple red to sky blue.

CALCULATION

Weight of the sample taken = W g

Volume of hydrochloric acid extract prepared =500 ml

Volume of hydrochloric acid extract pipette out for R_2O_3 estimation =50 ml

Volume of R_2O_3 filtrate made upto = 250 ml

Volume of R_2O_3 filtrate pipetted out for calcium estimation =10 ml

Volume of 0.02 N EDTA used for calcium and magnesium = a ml

Volume of 0.02 N EDTA used for calcium alone = b ml

Volume of 0.02 N EDTA used for magnesium alone = (a – b) ml

1 ml of 0.02 N EDTA = 0.0004 g calcium

1 ml of 0.02 N EDTA = 0.0004 g magnesium

Percentage of calcium on moisture free basis

$$= 0.0004 \times b \times \frac{250}{10} \times \frac{500}{50} \times \frac{100}{W} \times \frac{100}{(100\text{-}M)}$$

Percentage of magnesium on moisture free basis

$$= 0.00024 \times (a - b) \times \frac{250}{10} \times \frac{500}{50} \times \frac{100}{W} \times \frac{100}{(100-M)}$$

M = Moisture basis

APPENDIX- VIII

ESTIMATION OF PROTEIN (Lowry *et al.* 1951)

Principle

The blue colour developed by the reduction of the phosphomolybdic-phospho tungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartrate are measured in the Lowry's method.

Materials

2 % sodium carbonate in 0.1 N sodium hydroxide (Reagent A). 0.5 % copper sulphate ($CuSO_4$. $5H_2O$) in 1% potassium sodium tartrate (Reagent B). Alkaline copper solution: 50 ml of reagent A and 1 ml of reagent B were mixed prior to use (Reagent C). Folin-Ciocalteau reagent (Reagent D). Protein solution (stock standard): Weighed accurately 50 mg of bovine serum albumin (fraction V) and dissolved in distilled water and made up to 50 ml in a standard flask.

Working standard

10 ml of the stock solution was diluted to 50 ml with distilled water in a standard flask. 1ml of this solution contains 200 µg protein.

Procedure

Extraction of Protein from Sample

Extraction is carried out with buffers used for the enzyme assay. About 50 mg of the sample was taken and ground well with a pestle and mortar in 5-10 ml of the buffer and centrifuged. The supernatant was used for protein estimation.

Estimation of Protein

A quantity of 0.2, 0.4, 0.6, 0.8 and 1 ml of aliquots of the working standard were pipetted into a series of test tubes 0.1 ml and 0.2 ml of the sample extract in two other test tubes. The volume was made up to 1 ml in all test tubes. A test tube with 1 ml of water served as the blank. 5 ml of reagent C was added to each tube including the blank, mixed well and allowed to stand for 10 minutes. Then, 0.5 ml of reagent D was added, mixed well and incubated at room temperature in the dark for 30 minutes. Blue colour developed was read in a spectrophotometer (UV-vis Spectrophotometer model 108, Systronics, India). A standard graph was drawn and the amount of protein in the sample was calculated.

CALCULATION

Expressed the amount of protein mg/gm or 100 g sample

$$= \frac{\text{mg of protein}}{\text{volume of test standard}} \text{ x conc. of the standard}$$

APPENDIX- IX

ESTIMATION OF CARBOHYDRATE (Hedge and Hofreiter, 1962)

(Anthrone Method)

Principle

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This compound forms a green green colour in a dilute solution and a blue color in a concentrated solution. This compound forms a green colored product with an absorption maximum at 630 nm.

Materials

2.5 N HCl, Anthrone reagent: 200 mg anthrone was dissolved in 100 ml of ice cold 95% H_2SO_4 and it was prepared fresh before use. Standard glucose: (Stock) 100 mg of glucose was dissolved in 100 ml water. Working standard: 10 ml of stock solution was diluted in 100 ml distilled water and stored in a refrigerator after adding a few drops of toluene.

Procedure

100 mg of the sample (leaf) was taken in a boiling tube with 5 ml of 2.5 N HCl, hydrolyzed by keeping it in a boiling water bath for three hours and cooled to room temperature. Then, it was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifuged. The supernatant was collected and 0.5 and 1 ml aliquots were taken for analysis. From the working standard, the standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml and '0' served as blank. The volume was made up to 1 ml in all the test tubes including the sample test tubes by adding distilled water. Then, 4 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath. Then, it was cooled rapidly and the green colour developed was read at 630 nm. A standard graph was drawn by plotting concentration of the standard on the axis versus absorbance on the y-axis. From the graph, the amount of carbohydrates

present in the sample was calculated.

CALCULATION

Amount of carbohydrate present in 100 mg of the sample

$$= \frac{\text{mg of glucose}}{\text{volume of test sample}} \times 100$$

APPENDIX- X

ESTIMATION OF CHLOROPHYLL (Arnon, 1949)

Principle

Chlorophyll was extracted in 80% acetone. The absorption at 663 nm, 645 nm and 652 nm were read in a spectrophotometer using the absorption coefficients and the amounts of chlorophyll contents were calculated.

Materials

Analytical grade acetone was diluted to 80 % acetone (prechilled).

Procedure

Accurately weighed 1 g of finely cut and well mixed representative leaf sample. It was ground to a fine pulp with the addition of 20 ml of 80% acetone with a mortar and pestle and was centrifuged as 5,000 rpm for 5 minutes. The supernatant was transferred to a 100 ml volumetric flask. The residue was ground with 20 ml of 80% acetone, centrifuged and the supernatant was transferred to the same volumetric flask. This procedure was repeated until the residue was colourless. The mortar and pestle were also washed thoroughly with 80% acetone and the washing was collected in the volumetric flask. The volume was made up to 100 ml with 80% acetone. The absorbance of the solution was read at 645, 663 and 652 nm against the solvent (80% acetone) blank.

CALCULATION

The amount of chlorophyll present in the extract was calculated in mg chlorophyll g^{-1} tissues by using the following equations.

$$\text{Chlorophyll 'a' mg } g^{-1} \text{ tissues} = 12.7\, A_{(663)} - 2.69\, A_{(645)} \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll 'b' mg } g^{-1} \text{ tissues} = 22.9\, A_{(645)} - 4.68\, A_{(663)} \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll mg } g^{-1} \text{ tissue} = 20.2\, A_{(645)} + 8.02\, A_{(663)} \times \frac{V}{1000 \times W}$$

Where,

A = absorbance of specific wavelengths

V = final volume of chlorophyll extract in 80% acetone.

W = fresh weight of tissue extract

APPENDIX- XI

ESTIMATION OF LEGHAEMOGLOBIN CONTENT

(Appleby and Bergersen, 1980)

Principle

Haemoglobin reacts with pyridine in strong alkali to produce hemochrome. The hemochrome is measured at 556 nm.

Reagents

Diluent buffer: Sodium (0.1 M) / Potassium phosphate buffer (pH 7.4). Alkaline pyridine reagent: Dissolved 0.8 g NaOH in 50 ml water and cool. Added 33.8 ml of pyridine (33.2g), dissolved and diluted to 100 ml with water. This produces 4.2 M pyridine in 0.2 M NaOH. Sodium Dithionate: Ground finely and stored in small stoppered tubes in desiccator. Potassium Hexacyanoferrate.

Procedure

Extraction: Fresh or thawed nodules were mixed with 1-3 volumes of phosphate buffer and macerated in a mixer. It was filtered through two layers of cheese cloth. The nodules debris was discarded. The turbid reddish-brown filtrate was clarified by centrifuging at 10,000 rpm for 10-30 minutes diluted suitably. To a suitable volume (2-5 ml) of the extract, an equal volume alkaline pyridine reagent was added and mixed well. The solution becomes greenish-yellow due to the formation of ferric hemochrome. The hemochrome was taken in equal quantity in two tubes. To one portion, few crystals of sodium dithionate was added to reduce the hemochrome and stirred well without aeration. The absorbance was measured at 556 nm after 2-5 minutes against a reagent blank in a spectrophotometer. To the other portion, a few crystals of potassium hexacyanoferrate was added to oxidize the hemochrome and read at 539 nm in a spectrophotometer after 2-5 minutes against a reagent blank.

CALCULATION

$$\text{Lb concentration (mM)} = A_{556} - A_{539} \times 2D/23.4$$

Where, D is the initial dilution.

(The calculation is based upon the equation $E = 23.4 \times 103\ mol^{-1}\ cm^{-1}$)

APPENDIX – XII

ESTIMATION OF AVAILABLE NITROGEN IN SOIL (Subbiah and Asija, 1956) (Alkaline Permanganate Method)

Principle

A known weight of soil is mixed with excess of alkaline permanganate and distilled organic matter present in soil is oxidized by the nascent oxygen liberated by $KMnO_4$ in the presence of NaOH and thus ammonia is released. This released ammonia is absorbed in a known volume of boric acid (2%) containing double indicator and converted to ammonium borate. This ammonium borate is titrated against standard H_2SO_4.

Reagents

0.32% KMnO4 solution (3.2 g of $KMnO_4$ dissolved in one litre of distilled water). 2.5% NaOH solution (25 g of NaOH dissolved in one litre of distilled water). 2% boric acid (20 g of boric acid dissolved in one litre of distilled water). N/50 H_2SO_4 (30 ml of Conc. H_2SO_4 is diluted to one litre with distilled water and standardized by titration with N/10 Na_2CO_3. This gives N/10 H_2SO_4. From this N/50 H_2SO_4 is prepared by dilution. Double indicator bromocresol green (0.5 gm) and methyl red (0.1 g) dissolved in 100 ml and ethyl alcohol.

Procedure

Weighed 20 g of soil and transferred into a distillation flask. Added 30 ml of distilled water to moist the soil and 1 ml of liquid paraffin. Added few pieces of glass beads to avoid frothing. Added 100 ml of freshly prepared 0.32% $KMnO_4$ and 100 ml 2.5% NaOH to the soil in the distillation flask. A 100 ml beaker containing approximately 20 ml of 2% boric acid with double indicator was kept below the delivery end of the condenser in the distillation set. Distilled the contents and the liberated ammonia was collected in boric acid. Distillation continued until the release of ammonia. Titrate the ammonia collected in boric acid with N/50 H_2SO_4.

CALCULATION

Weight of the soil taken = 20 g

Volume of N/50 H_2SO_4 = X ml (titre value)

1 ml of N/10 H_2SO_4 = 0.0014 g N

Therefore 1 ml of N/50 H_2SO_4 = 0.00028 g N

X ml of N/50 H_2SO_4 = 0.00028 × X g N

This is present in 20 g of soil

Therefore, N present in Kg/Ha = $0.00028 (X/20) \times 10^6$

APPENDIX - XIII

ESTIMATION OF AVAILABLE PHOSPHORUS IN SOIL (Jackson, 1973)

(Calorimetry Method)

Principle

The combination of HCl and NH_4F extracts acid soluble forms of phosphorus such as mono calcium phosphate. The fluoride ion has the special property of complexing Al^{+++} and Fe^{+++} in acid solution with consequent release of phosphorus held in the soil by these ions. The phosphorus so released into the soil solution in estimated calorimetrically as available phosphorus.

Reagents

NH_4F solution (1N): 37 g of NH_4F was dissolved in 1 litre of distilled water. HCl (0.05N): 20.2 ml conc. HCL diluted 500 ml with distilled 500 ml with distilled water. Bray No. 1 extractant [0.03 NH_4F and 0.02 N HCL]: 15 ml of 1N NH_4F and 25 ml of 0.5N HCL are mixed and the volume was upto 500 ml with distilled water. Ascorbic acid.

Procedure

Weighed 5gof soil and transfer to a 100 ml polythene shaking bottle. Added 50 ml of Bray 1 extractant. Shake the contents in a reciprocator mechanical shaker for one minute. Filtered the contents through Whatman No. 40 filter paper. Simultaneously conducted a blank. Pipetted out 5 ml of filtrate into 25 ml volumetric flask. Added 4 ml of reagent B as in Olsen's method and made up the volume to 25 ml. The intensity of the colour developed was measured in a photoelectric calorimeter using filter (660 nm).

CALCULATION

Weight of soil taken = 5 g

Volume of $NaHCO_3$ = 50 ml

Volume of extractant solution used for Phosphorus estimation = 5 ml

Calorimeter reading = T

Concentration of phosphorus read from standard graph for the reading T = X ppm

= X mg/ml

= $X/10^6$ gm/ml

Therefore in 25 ml of solution = $X/10^6 \times 25$ g

This is present in 50 ml of the extractant solution and 5 g of soil

Therefore available P_2O_5 in kg/ha = $X \times 25 \times 50 \times 2 \times 10^6 \times 5 \times 5$

APPENDIX – XIV

ESTIMATION OF AVAILABLE POTASSIUM IN SOIL

(Standford and English, 1949)

(Flame Photometry Method)

Principle

The potassium ions in the exchange site are replaced with NH_4^+ and K^+ which is released. The concentration of K ions in the solution is then determined using flame photometer.

Reagents

1 N Ammonium acetate (Neutral in pH): Dissolved 77 g of AR grade ammonium acetate in 1000 ml distilled water. pH adjusted to 7.0.

Procedure

Transferred 5 g of soil into a polythene shaking bottle. Added 25 ml of 1 N ammonium acetate and contents shaked in a mechanical reciprocating shaker for 5 minutes.

Contents filtered through Whatman No. 40 filter paper. Filtrates were fed into the flame photometer and the readings recorded. Using standard curve available potassium content was calculated.

CALCULATION

Weight of the soil taken	= 5 g
Volume of the extractant used	= 25 ml
Flame photometer reading	= T
Concentration of K in the standard curve	= X ppm
	= X mg/ml
	= $X/10^6$ g/ml
Therefore in 25 ml solution	= $X/10^6 \times 25$ g
This is present in 5 g of soil	
Therefore available K in soil in kg/ha	= $X/10^6 \times 25 \times 2 \times 10^6/5$

Annexures

भारतसरकार
GOVERNMENT OF INDIA
पर्यावरण,वनऔरजलवायुपरिवर्तनमंत्रालय
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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2023-24/Tech - 451 दिनांक/Date: 18th May 2023

पादप प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen given by you for authentication is identified as ***Abelmoschus esculentus*** (L.) Moench - **MALVACEAE.**

अभिनिर्धारित प्रतिरूप को संबंधित कॉलेज/विभाग/संस्थान के पादपालय में परिरक्षण हेतु वापस किया जाता है।/ The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

18/5/2023

डॉ. एम. यु. शरीफ़/Dr. M. U. SHARIEF
वैज्ञानिक 'एफ' एवं कार्यालयाध्यक्ष/
SCIENTIST 'F' & HEAD OF OFFICE

सेवा में / To

Mrs. V. KARTHIYA
Ph.D. Research Scholar
Department of Botany
Avinashilingam Institute for Home Science and
Higher Education for Women
COIMBATORE – 641 043

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bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2023-24/Tech - 452 दिनांक/Date: 18th May 2023

पादप प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen given by you for authentication is identified as ***Cyamopsis tetragonoloba*** (L.) Taub. - **FABACEAE.**

अभिनिर्धारित प्रतिरूप को संबंधित कॉलेज/विभाग/संस्थान के पादपालय में परिरक्षण हेतु वापस किया जाता है।/ The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

18/5/2023

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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2023-24/Tech – 450 दिनांक/Date: 18th May 2023

पादप प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen given by you for authentication is identified as ***Coriandrum sativum*** L. **- APIACEAE.**

अभिनिर्धारित प्रतिरूप को संबंधित कॉलेज/विभाग/संस्थान के पादपालय में परिरक्षण हेतु वापस किया जाता है।/ The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2023-24/Tech – 453 दिनांक/Date: 18th May 2023

पादप प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen given by you for authentication is identified as ***Trigonella foenum-graecum*** L. - **FABACEAE.**

अभिनिर्धारित प्रतिरूप को संबंधित कॉलेज/विभाग/संस्थान के पादपालय में परिरक्षण हेतु वापस किया जाता है।/ The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

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Higher Education for Women
COIMBATORE – 641 043

Publications

Avinashilingam Institute for HomeScience and Higher Education for Women
(Deemed to be University Estd. u/s 3 of UGC Act 1956, Category 'A' by MHRD
Re-accredited with A++ Grade by NAAC. CGPA 3.65/4, Category I by UGC
Coimbatore - 641 043, Tamil Nadu, India

Appendix L2
(Item No 5 of Check List)
Details of Research Publications

S. No	Article	Journal	Other Details Vol/No/Page No/ Year	Published in UGC- CARE / Scopus Indexed/ Web of Science
1	First report on toddy palm shell-based vermicompost by *Eisenia fetida*	International Journal of Environmental Science and Technology	20(10); 11061-11074; (2023)	Scopus Web of Science
2	Groundnut shells and toddy palm shells recycling through vermicomposting technology and its efficacy on growth and yield attributes of cluster bean (*Cyamopsis tetragonoloba* L.) Taub.	Current Agriculture Research Journal	11(1); 297-305; (2023)	UGC CARE list Group I

*Proof of list of Journals from Internet to be attached along with copies of reprints.

Scholar : 5/10/23

Supervisor : A. Vijayalakshmi 5/10/23

Checked By: A. Vijayalakshmi 6/10/23

HoD/~~Dean of Respective School~~

The scholar Miss. Kavithiya, V (17PHBOF004) has published her article in the following journals:

1. International Journal of Environmental Science and Technology - is indexed and active in Scopus from 2005 to present. Her article was published in Vol. 20, No. 10, ~~oct~~ Pg. 11061 - 11074, October, 2023 and
2. Current Agriculture Research Journal - is indexed and active in UGC Care list Group I from July 2022 to present. She published her article in Vol. 11, No. 1, April, 2023 Pg. 297 - 305.

J. Jayabalan

05.10.23.

International Journal of Environmental Science and Technology (2023) 20:11061–11074
https://doi.org/10.1007/s13762-022-04597-8

ORIGINAL PAPER

First report on toddy palm shell-based vermicompost by *Eisenia fetida*

K. Velmurugan[1] · V. Annamalai[1]

Received: 23 July 2021 / Revised: 24 May 2022 / Accepted: 10 October 2022 / Published online: 17 October 2022

Abstract
Disposable groundnut shells and toddy palm shells are a threat to the environment. A significant amount of shell residuals dumping or burning process leads to the accumulation of waste causing environmental pollution. Hence, minimizing the waste by recycling promotes zero-waste production. The present study toward the degradation of GNS and TPS through the microbial consortium and *Trichoderma asperelloides* along with vermicomposting technology has been presented. During the composting bacterial, fungal and actinomycetes counts were observed at the regular interval of 30, 60 and 90 days which achieved peak stage on 60th day of composting. Further, physical and chemical parameters of raw and composted samples were analyzed. The results observed range between pH (6.01–6.40), EC (1.39–2.59 ds m^{-1}), TN (0.91–1.39%), TP (1.98–2.51%), TK (2.36–3.85%), TCa (2.27–3.25%) and TMg (2.18–2.99%), were significantly increased while lignin (9.54–6.69%), cellulose (10.71–6.35%) OC (29.8–26.5%) and C:N ratio (29:1–19:1) were considerably reduced. In addition, FT-IR analysis shows the high degradation in C_3 and C_6 the same result ensured by XRD analysis. All these analyses reflect the quality of organic fertilizer with the help of microbial consortium in a short period. Hence, the present study highlights the organic fertilizer from GNS and TPS through vermicomposting technology minimizing the waste simultaneously protecting the environment.

Editorial responsibility: Nour Sh. El-Gendy.

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Graphical Abstract

Keywords Groundnut shell · Microbial consortium · FT-IR · XRD and waste management

Introduction

Dumping of solid waste is a prominent issue worldwide. The accumulation of waste is generated gradually by different human activities such as industrial, domestic, agricultural and municipal solid waste which are disposed of by landfilling, burning or buried. Those wastes are causing health hazards to human beings and lead to environmental pollution. In its order agricultural wastes GNS - Groundnut shell and TPS - Toddy palm shell plays an important role in waste management. Groundnut is a prominent leguminous crop grown mainly for oil and seed worldwide. GNS are leftover products obtained after the removal of groundnut seed from its pods which degrade very slowly under natural conditions (Zheng et al. 2013). Consumption of groundnut is tremendous in the world particularly in China (171.50 lakh tones), India (91.79 lakh tones), United States (32.81 lakh tones), Nigeria (24.20 lakh tones) and Sudan (16.41 lakh tones) in 2017–18. Annually around 11,000,000 tons of GNS waste have been released from the peanut industry worldwide.

Toddy palm (*Borassus flabellifer* L.) is Tamil Nadu's state tree that belongs to the family Arecaceae. In 2002, there are about 140 million toddy palms distributed worldwide (Naguleswaran et al. 2010). In Sri Lanka (10 million), India (60 million), Burma (2.5 million), Cambodia (1.8 million), Indonesia (0.5 million) etc., Recently Krishnaveni et al (2020) reported that India has around 102 million toddy palms present (Tamil Nadu, Andhra Pradesh, Odisha, West Bengal, Bihar, Karnataka and Maharashtra). It is an economically important crop; only the discarded part was their shell which consists of 40–50% of mesocarp and exocarp (shell) (Rodiah et al. 2019). Fifty percent of fruit is discarded after the processing, the usual way to dispose of TPS by landfilling causing environmental pollution. TPS takes a very long period for degradation due to wetness and bulkiness which are the most favorable condition for mosquito

breeding, create dangerous disease-like Chikungunya, dengue, and malaria.

In order to overcome this problem recycling is the best approach to utilize and minimize waste. Only a few researchers handle those waste for recycling to make a useful product like the production of biodiesel Udeh (2018), bioethanol (Tejas et al. 2017), hydrogen (Evans et al. 2002), growth media (Jalal et al. 2017), paper production Upendra et al (2018) and dye degradation (Zakariyya and Saifullahi 2018) from GNS waste. On the other hand, TPS into the production of biofuel (Madhu et al. 2016), activated carbon (Khaing et al. 2014), building material (Osakwe et al. 2015), charcoal briquettes (Utchariyajit et al. 2019), cellulose microfibers (Reddy et al. 2016), biochar (Kongnine et al. 2020) etc. However, higher level of lignin and cellulose content present in GNS and TPS waste is a major disadvantage of its use on a large scale. Among the various solutions, compost technology got major attention to deal with waste to convert wealth which is the easiest method for composting and low-cost production. However, GNS was not much utilized and TPS was not utilized still to date in the compost technology to convert efficient organic fertilizer even it has rich carbon and nitrogen sources. Nowadays, the circulation of chemical fertilizer has gradually increased which affects the soil and human health. So, organic fertilizers got many recommendations to enhance plant growth and soil health without affecting the environment.

In the present study, GNS and TPS degrade with the help of *Trichoderma asperelloides*, microbial consortium (*Bacillus licheniformis* (MTCC-10498), *Paecilomyces variotti* (MTCC-6581), *Streptomyces lavendulae* (MTCC-6821), *Pleurotus florida* (MTCC-9194) and the epigeic earthworm *Eisenia fetida* were used pit composting method in December 2019 at Avinashilingam institute for home science and higher education for women, Coimbatore, Tamil Nadu, India. It is the very easiest method for composting and low-cost production. The advantages of pit composting are decomposed very faster and obtained good quality manure; only the disadvantage is high labor demand and not suitable for the rainy season. The earthworm *Eisenia fetida* is a surface feeder more suitable for vermicomposting and also known as a red worm, tiger worm, red wiggler etc. it consumes half of its body weight per day and high reproductive rate. Vermicompost contains an enormous amount of available nutrients such as nitrogen, phosphorus, potassium (Edwards and Burrows 1988), humic acids (Senesi et al. 1992), enzymes, vitamins (Ismail 1997) and also plant growth promoting hormones such as auxins, gibberellins and cytokinins (Krishnamoorthy and Vajrabhiah 1986).

The current study provides six different combinations of compost made from GNS and TPS such as C - Control, C_1 (GNS + *Trichoderma asperelloides* + *Eisenia fetida)*, C_2 (GNS + microbial consortium), C_3 (GNS + microbial consortium + *Eisenia fetida),* C_4 (TPS + *Trichoderma asperelloides* + *Eisenia fetida),* C_5 (TPS + microbial consortium), C_6 (TPS + microbial consortium + *Eisenia fetida*), respectively. Organic fertilizer-like composts and vermicompost promotes soil fertility, improve the structure of soil (Follet et al. 1981), increases microbial population (fungi, bacteria and actinomycetes) (Edwards 1998; Tomati et al. 1987), diversity (Barakan et al. 1995), (Zink and Allen 1998), and retain water content in the soil. The current study toward the degradation of GNS and TPS converted into valuable organic fertilizer through vermicomposting technology to achieve minimization of the waste.

Materials and methods

Collection of materials

GNS was collected from groundnut field Pollachi and TPS were collected from roadside areas in and around Coimbatore location, which were cut into small pieces and dried under sunlight for one week to eliminate their moisture content. Both the samples have taken a long period for decomposition due to the presence of the high amount of lignin and cellulose content. The selected microorganisms such as *Bacillus licheniformis, Paecilomyces variotti,* (*Streptomyces lavendulae*, *Pleurotus florida* were collected from the Institute of Microbial Technology, Chandigarh and *Trichoderma asperelloides* was collected from Tamil Nadu Agricultural University, Coimbatore. The epigeic earthworm *Eisenia fetida* commonly known as tiger worm collected from the earthworm breeding center at Kisan Vigyan Kendra Coimbatore.

Preparation of microbial consortium

The selected microorganisms like Bacteria (*Bacillus licheniformis*), Cellulolytic fungi (*Paecilomyces variotti*), lignolytic fungi (*Pleurotus florida*) and Actinomycetes (*Streptomyces lavendulae*) were used to the prepared microbial consortium. Bacterial, fungal and actinomycete isolates were subcultured from the mother culture and approximately 50 ml of culture was inoculated into 500 ml of respective culture medium. Nutrient agar medium for bacteria, potato dextrose agar medium for fungi and kenknights & Munaier's medium for actinomycetes incubated at 35 °C, 25 °C and 30 °C for 1 day, 3 days and 7 days, respectively. Further, the equal amount of microbial biomass 1:1:1 ratio (bacteria, fungi and actinomycetes) approximately 100 g of cultures were mixed with cow dung slurry with the ratio of 1 kg of cow dung in 10 L of water kept at room temperature for 1 day.

Experimental setup

The compost was prepared at Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India located at 76°56′44.27″ E longitude and 11°1′11.14″ N latitude. The study consists of six composting pits C_1, C_2, C_3, C_4, C_5 and C_6 (C standard for compost) in the aerobic condition, each pit was constructed by 2 feet height and 1.5 feet width. Five kilograms of sundried GNS was transferred into C_1, C_2, C_3 and TPS was transferred into C_4, C_5 and C_6 composting unit along with cow dung. 20 g of *Trichoderma asperelloides* was added to C_1, C_4 and 100 g of microbial consortium mixed with cow dung slurry were sprayed into C_2, C_3, C_5 and C_6 uniformly spread over like sandwich. The compost moisture content was maintained by sprinkling about 50% of water to prevent drying and it was mixed thoroughly once a week. After 30 days the compost was partially decomposed while C_1, C_3, C_4 and C_6 composts were transferred to the plastic trays measuring 50 cm length, 35 cm width and 15 cm depth. 20 healthy epigeic earthworms (*Eisenia fetida)* were inoculated into the plastic trays containing respective samples. The trays were kept in a shade area for 60 days, maintaining moisture content around 80% (Fig. 1).

Enumeration of bacteria, fungi and actinomycetes

One gram of sample was taken in sterile conical flask containing 9 ml of distilled water, shaken well for 30 min in vortex mixer. Using different sterile pipettes, 1 ml of 10^6 and 10^4 serial dilutions of the samples were inoculated into sterile Petri dishes containing Nutrient agar medium for bacteria, Potato dextrose agar medium for fungi and kenknights & Munaier's medium for Actinomycetes, incubated for 1 day, 3 days and 7 days, respectively. Microbial colonies were counted during the decomposition of GNS and TPS at regular intervals of 30, 60 and 90 days. Viable colonies were counted with the help of the colony counter.

$$\text{Colony forming unit} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of petri dish}}$$

Physicochemical analysis

The air dried, finely ground homogenous samples were passed through a 2 mm sieve, analyzed by the following methods. pH and Electrical conductivity were determined by pH meter and conductivity bridge in the ratio of sample and water (1:10 w v^{-1}), respectively. Lignin was

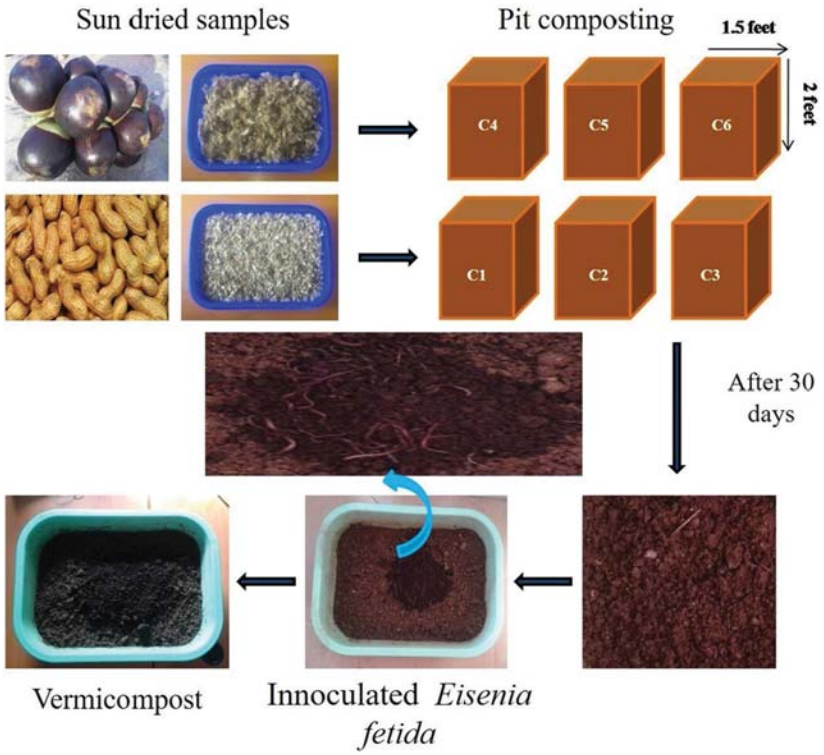

Fig. 1 Schematic diagram of the vermicomposting process of GNS and TPS waste

analyzed by acid detergent fiber (ADF) and acid detergent lignin (ADL) (Goering and Soest 1975) Cellulose (Updegroff 1969), Organic carbon was analyzed by wet chromic acid oxidation method (Walkley and Black 1934), Total nitrogen by micro kjeldahl method (Humphries 1956), Total potassium by flame photometer method, Total phosphorus (Jackson 1973), Total calcium and Total magnesium by versanate method (Jackson 1973).

Fourier transform-infrared spectroscopy

FT-IR spectra of raw (GNS and TPS) and composted (C_1, C_2, C_3, C_4, C_5 and C_6) samples were dried at room temperature for one day to eliminate moisture content. Finely ground 2 mg of homogeneous sample was mixed with 400 mg of Potassium Bromide and compressed into pellets and recorded the transmittance response from 4000 to 400 cm^{-1} on a Shimadzu FT-IR 8400S spectrometer. The FT-IR spectra of GNS and TPS were compared with their processed composts (C_1, C_2 and C_3) and (C_4, C_5 and C_6), respectively.

X-ray diffraction method

In extension of compost degradation study after FT-IR analysis, X-Ray diffraction analysis was used to ensure the degradation of C_3 & C_6 by physical and chemical structural changes. The same dried sample was used in FT-IR and XRD analysis. The samples were prepared by grinding them into fine powder such as C_3 and C_6 compared with GNS and TPS analyzed by using Empyrean nano edition.

Statistical analysis

Enumeration of the microbial population was concluded from triplicate values and the data were subjected to statistically analyze by one-way ANOVA. Preparation of graphs and tables was done by Microsoft Excel 2007.

Results and discussion

The present study enumerates the total microbial population of composted GNS (C1, C2 & C3) and TPS (C4, C5 & C6) through the vermicomposting process on 30, 60 and 90 days is presented in Table 1. The highest microbial load was observed on 60 days of the composting during the thermophilic stage promotes maximum colonies shown in Fig. 2.

Population of bacteria

At the beginning of the composting process, about 0–30 °C mesophilic bacteria appeared prominently and the maximum count was found in C_6 (1.92×10^6 CFU g^{-1}) followed by C_3 (1.76×10^6 CFU g^{-1}) compared to the C (1.12×10^6 CFU g^{-1}) on 30 days at initial phase due to the degradation of GNS and TPS with the help of microbial consortium. Early stage of the composting process mesophilic bacteria being reproduced by the activity of breakdown of nitrogen and carbon in the organic materials afterward which was completely disappeared within a few days or a week and thermophilic bacteria started to thrive. During the middle phase thermophilic bacteria occupied 60% of the population in the composting site due to the generation of high temperature in composting pit about < 40 °C. Only thermophilic bacteria appeared on this stage with the highest counts were obtained from C_6 (2.48×10^6 CFU g^{-1}) followed by C_5

Table 1 Colony forming unit of Bacteria, Fungi and Actinomycetes during the composting and vermicomposting

Treatments	Bacteria			Fungi			Actinomycetes		
	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
C	1.12 ± 0.16	1.62 ± 0.04	0.88 ± 0.12	0.26 ± 0.08	0.42 ± 0.03	0.21 ± 0.05	0.48 ± 0.06	0.58 ± 0.03	0.26 ± 0.06
C_1	1.52 ± 0.08	1.96 ± 0.24	1.24 ± 0.04	0.48 ± 0.06	0.64 ± 0.11	0.32 ± 0.03	0.53 ± 0.08	0.69 ± 0.05	0.34 ± 0.08
C_2	1.68 ± 0.16	2.08 ± 0.04	1.56 ± 0.16	0.64 ± 0.11	0.85 ± 0.03	0.53 ± 0.13	0.64 ± 0.11	0.77 ± 0.11	0.48 ± 0.11
C_3	1.76 ± 0.12	2.24 ± 0.16	1.60 ± 0.20	0.74 ± 0.16	0.96 ± 0.03	0.58 ± 0.06	0.77 ± 0.13	0.85 ± 0.05	0.58 ± 0.11
C_4	1.32 ± 0.12	2.17 ± 0.17	1.12 ± 0.32	0.40 ± 0.08	0.53 ± 0.27	0.32 ± 0.06	0.61 ± 0.08	0.74 ± 0.11	0.32 ± 0.03
C_5	1.48 ± 0.20	2.32 ± 0.16	1.36 ± 0.16	0.58 ± 0.06	0.74 ± 0.11	0.53 ± 0.03	0.58 ± 0.03	0.80 ± 0.14	0.42 ± 0.14
C_6	1.92 ± 0.28	2.48 ± 0.08	1.44 ± 0.20	0.85 ± 0.05	0.90 ± 0.11	0.66 ± 0.08	0.69 ± 0.16	0.90 ± 0.11	0.53 ± 0.16
SEd		0.13807			0.07960			0.08047	
CD ($P < 0.05$)		0.27871			0.16067			0.16244	

C Control, C_1 (GNS + *Trichoderma asperelloides* + *Eisenia fetida*), C_2 (GNS + microbial consortium), C_3 (GNS + microbial consortium + *Eisenia fetida*), C_4 (TPS + *Trichoderma asperelloides* + *Eisenia fetida*), C_5 (TPS + microbial consortium), C_6 (TPS + microbial consortium + *Eisenia fetida*)

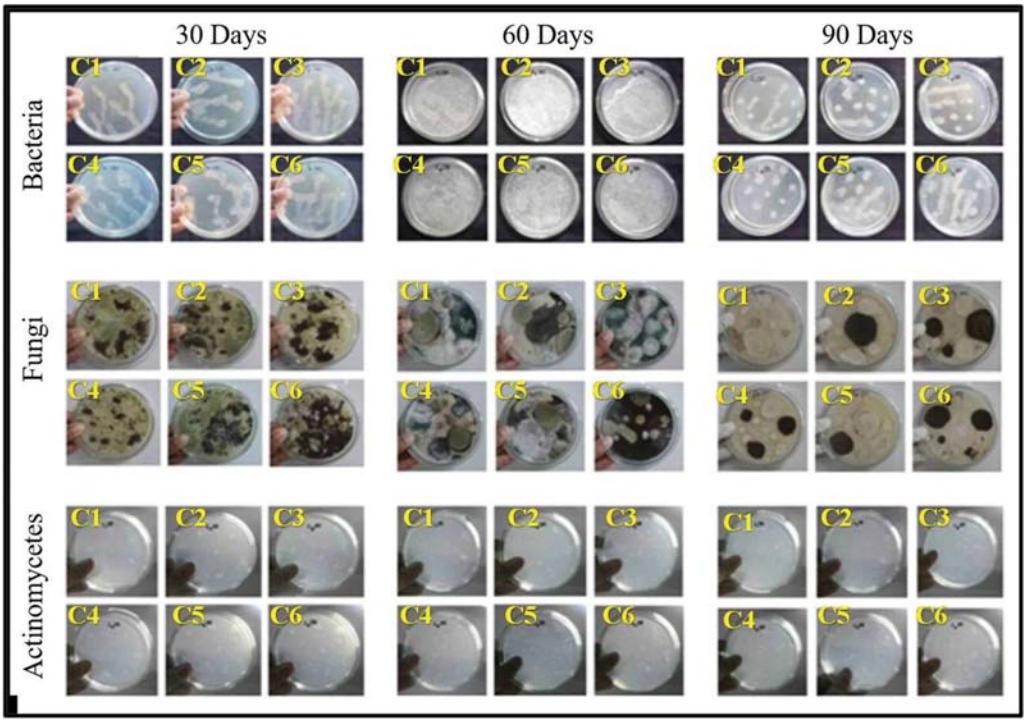

Fig. 2 Microbial population of GNS (C_1, C_2, C_3) and TPS (C_4, C_5, C_6) compost on 30, 60 and 90 days

(2.32×10^6 CFU g^{-1}) on 60 days, respectively. Further, at end of the composting (cooling or maturation phase) bacterial colonies were slightly decreased than a thermophilic stage. However, during the mesophilic, thermophilic and maturation phase increase in bacterial counts was predominant in C_6 may be due to good degradation of substrates by microbial consortium and source of energy provided by *Eisenia fetida.* Senapati and Dash (1984) revealed that the increasing the bacterial population in the vermicompost may be attributed to the good nutritional supply from the earthworm gut.

Population of Fungi

Maximum fungal growth was observed at the mesophilic phase due to the attributes of high moisture content present in the composting unit (Srivastava et al. 2011) and more colonies have appeared in a moist environment than the dry environment. The fungal population dominates at the middle phase of composting with a count of C_3 (0.96×10^4 CFU g^{-1}) followed by C_6 (0.90×10^4 CFU g^{-1}) compared to the control which might be due to the dual action of microbial consortium and earthworm. During the 60 days of composting thermophilic fungi promotes the maximum number of colonies simultaneously mesophilic fungi was completely disappeared. The result has coincided with the findings of Hefnawy et al. (2013) the thermophilic fungi which are *Aspergillus, Humicola, Talaromyces,* and *Thermomyces* species isolated from the heating phase of the composting increased the fungal count. On the 90th day maximum colonies were found in C_6 (0.66×10^4 CFU g^{-1}) may be good degradation and energy provided from the earthworms. Aira et al. (2006) reported that the degradation of cellulose due to the activity of *Eisenia fetida* promotes fungal growth during the vermicomposting process.

Population of Actinomycetes

Actinomycetes are fungi-like bacteria that creates an earthy aroma of good compost which is capable to degrade most recalcitrant materials such as lignin and cellulose in agricultural waste composting (Limaye et al. 2017; Partanen et al. 2010). Among all the treatments, GNS decomposed with the help of microbial consortium + *Eisenia fetida* significantly registered a higher actinomycetes population 0.77×10^4 CFU g^{-1} in C_3 followed by 0.69×10^4 CFU g^{-1} in C_6 on 30 days of composting. During the thermophilic phase actinomycetes load reaches the peak stage noted in C_6 (0.90×10^4 CFU g^{-1}) greater than C_3 (0.85×10^4 CFU g^{-1}) compared to the control which might be due to the nutrients

supply from the compost. Miller (1992); Mitchell et al. (1992) reported that the increases of actinomycetes probably appear during the thermophilic stage of composting plays an important role in converting cellulose and hemicellulose into a more degradable substances like starch and sugar. The least actinomycetes population obtained with a count of 0.58×10^4 CFU g^{-1} in C_3 which also agrees with the work of Chander et al. (2018) who reported that lower temperature promotes the growth of actinomycetes.

pH

The value of pH in the final compost slightly increased than raw organic materials such as GNS and TPS. Among the treatments, maximum pH value was observed in C_6 (TPS + microbial consortium + *Eisenia fetida*) indicating the acidic nature of the substrates. An increase in pH value in the compost is mainly due to the breakdown of small chained fatty acid with the help of microbes (Tognetti et al. 2007) and it indicates the increase in ammonium contents in the composting materials (Jagadabhi et al. 2018; Singh et al. 2010).

Besides, the above neutral pH value reflects the alkalinity of substrate produced ammonium gas in the air which enhanced the harmful pathogenic bacteria in the atmosphere (Mandal et al. 2014). However, in this study pH of composted GNS and TPS have neutral results shown in Fig. 3a. According to Edwards and Bohlen (1996) near neutral values of pH promotes the availability of macronutrients in the vermicompost simultaneously, conversion of pH values of compost depending upon the dynamic and substrate (Lim et al. 2011) which agrees with current work.

Electrical conductivity

The EC reflects the salt concentration of organic amendment and also indicates the suitability and safety of vermicompost at agricultural land (Karthika et al. 2018). In this study, electrical conductivity gradually raised i.e., (Vermicompost) ranged between 1.39 and 1.47 (ds m^{-1}) in GNS and 2.55–2.59 (ds m^{-1}) in TPS composts than raw materials as shown in Fig. 3b which is mainly due to the breakdown of phosphate and ammonium ions in the substrate. According to Garg et al. (2006) salt concentrations (electrical conductivity) are dependent on liberally available minerals and ions from earthworms by ingestion and excretion. Excreta of worms contains different soluble salts in the available form of potassium, phosphate and ammonium by the activity of earthworms and microorganisms (Khwairakpam and Bhargava 2009). Lazcano et al. (2008) reported that the compost with EC value $< 3\mu S^{-1}$ is suitable for the safe disposal of the soil. A higher value of EC in the vermicompost might be unfavorable for the growth of earthworms (Ferna´ndez-Go´mez et al. 2010). However, in this study, EC value of six composts did not exceed $3\mu S^{-1}$ which indicates the compost prepared by GNS and TPS were more suitable for organic fertilizer.

Total organic carbon

Maximum reduction of organic carbon content was observed in C_6 (26.5%) followed by C_4 (26.9%) than raw materials. This may be due to the loss of C in the form of C_2O by microbial respiration during the composting (Pattnaik and Reddy 2010; Kale 1998). Karthika et al. (2018) also reported maximum loss of OC through vermicomposting. Usually, a higher reduction of total OC has observed in worm worked compost than worm without worked compost is also reported by Yuvaraj et al. (2019) and the combined application of earthworms and microorganisms decline the level of organic carbon (Lim et al. 2011). In the current study, the maximum reduction has occurred in both vermicompost GNS and TPS along with microbial consortia shown in Fig. 3c.

Total C:N ratio

Carbon nitrogen ratio is the important factor to determine the lusciousness of earthworms and the maturity & quality of the compost (Hendriksen 1990). Initial C:N ratio of GNS and TPS in this study were 38:1 and 40:1 presented in Table 2. The decline of C:N ratio in the substrate can be attributed to the loss of C as CO_2 and the addition of N through excretion of earthworms and respiration of microorganisms (Lim et al. 2011; Gajalakshmi and Abbasi 2002). After two months of the vermicomposting process, all the vermicompost substrates showed a considerable reduction on C_1, C_3, C_4. C_6 (with earthworm) than other compost C_2, C_5 (without earthworm). Among the treatments, maximum reduction of C:N ratio was noted in C_6 (19:1) followed by C_3 (21:1) by the dual action of microbial consortium and *Eisenia fetida* respectively as shown in Fig. 3d. Davidson et al. (1994), Khwairakpam and Bhargava (2009) also reports that less than 20% of C:N ratio enhanced the plant growth and compost maturity. Likewise, the present study, C_6 containing below 20% of C:N ratio showed may be successful results on the plant growth by enhancing soil condition as ideal organic fertilizer.

Total nitrogen

Nitrogen is a fundamental element required for successful plant growth (Liu et al. 2014) and the enhancement of total nitrogen in the vermicompost probably due to the conversion of ammonium nitrate to nitrate reported by Suthar and Singh (2008). Data shown in Table 2 reveals that the amount of total nitrogen in the raw GNS and TPS were up to 0.76% and

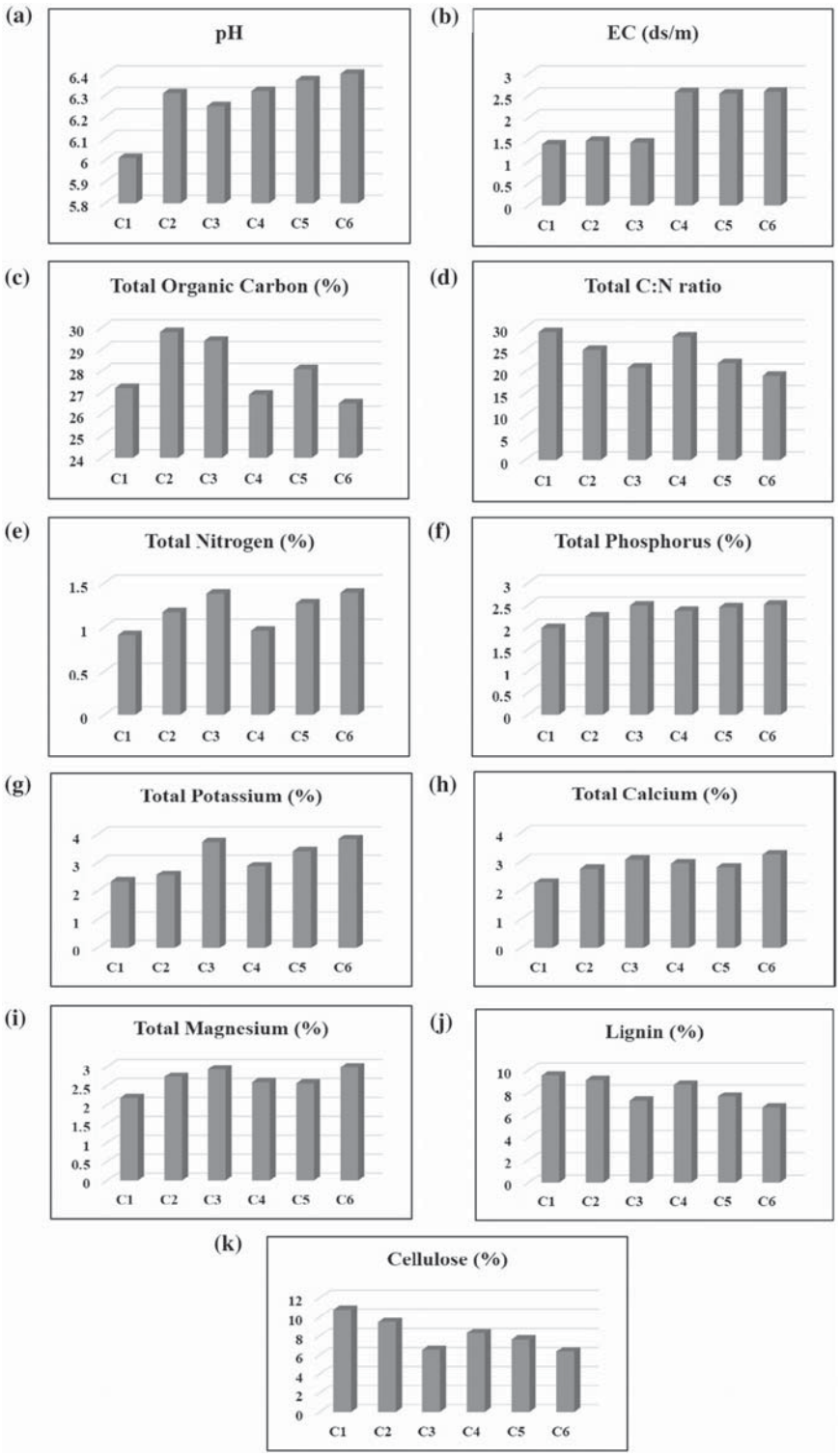

Fig. 3 Physicochemical properties of composted samples. C_1 (GNS + *Trichoderma asperelloides* + *Eisenia fetida*), C_2 (GNS + microbial consortium), C_3 (GNS + microbial consortium + *Eisenia fetida*), C_4 (TPS + *Trichoderma asperelloides* + *Eisenia fetida*), C_5 (TPS + microbial consortium), C_6 (TPS + microbial consortium + *Eisenia fetida*)

Table 2 Physicochemical properties of groundnut shell and toddy palm shell waste

Parameters	Groundnut shell	Toddy palm shell
pH	5.97	6.02
EC (ds m^{-1})	1.37	2.54
Total organic carbon (%)	32.7	36.5
Total C:N ratio	38:1	40:1
Total N (%)	0.86	0.91
Total P (%)	1.82	2.32
Total K (%)	1.21	1.57
Total Ca (%)	1.05	1.34
Total Mg (%)	0.98	1.24
Lignin (%)	28.5	37.4
Cellulose (%)	34.2	29.2

0.82%, respectively. During the decomposition of organic waste, the total nitrogen content was increased and this is due to the release of the nitrogenous substance from the earthworm gut through excreta. Maximum nitrogen content was obtained in C_6 (1.39%) and C_3 (1.38%) by the dual action of microbial consortium along with epigeic earthworm *Eisenia fetida* enhanced the process when compared to the other composts C_1 (0.91%), C_2 (1.17%), C_4 (0.96%) and C_5 (1.27%) presented in Table 3 and Fig. 3e. The result was coincident with the findings of Khomami et al. (2019) who stated that 100% peanut shells + azolla compost increased the total nitrogen content (2.8%) and an increasing trend of total nitrogen through vermicomposting was also reported by Plaza et al. (2007). Further, nitrogen fixing bacteria mineralize the carbon rich material thereby increasing the nitrogen content. *Bacillus cereus, B. megaterium, B. pumilus, B. licheniformis, B. circulans, B. firmus, B. brevis* and *B. subtilis* are nitrogen fixing bacteria based on nitrogenase activity (Xie et al. 1998). In this study, *B. licheniformis* was also found in the microbial consortium which promotes the increases of nitrogen through decomposition. A higher level of nitrogen content in the compost helps plants to produce more chlorophyll content (Jiayin et al. 2013).

Total phosphorus

Phosphorus is an important nutrient for photosynthesis and energy transfer within plants which improves flowering attributes and fruit growth in crop production (Lim et al. 2011). In this study, Fig. 3f shows that the maximum increases of total phosphorous content were observed in C_6 (2.51%) followed by C_3 (2.49%) compared to the other treatments which might be due to the attributes of earthworms and cow dung. C_6 and C_3 treatments have a higher quantity of cow dung along with a consortium of microorganisms and earthworms promoting total P content in the vermicompost. Availability of phosphorus increased from the activity of worm worked compost than worm without worked compost (Mitchell et al. 1981; Satchell and Martin 1984). Normally, cow dung contains a certain quantity of phosphorus content promotes the quality of the fertilizer (Arancon et al. 2004). According to Padmavathiamma et al. (2008) reported that source of Phosphatases released from the gut of earthworms converts insoluble phosphate into soluble phosphate with the help of P solubilizing microorganisms during the vermicomposting process, thus increasing total phosphorous in the compost.

Total potassium

As reported in Table 2 the total potassium in raw GNS and TPS was 1.21%, 1.57%, respectively. Maximum increases of

Table 3 Physicochemical characteristics of composting and vermicomposting of GNS and TPS through *Trichoderma asperelloides,* microbial consortium and *Eisenia fetida*

Parameters	C_1	C_2	C_3	C_4	C_5	C_6
pH	6.01	6.31	6.25	6.32	6.37	6.40
EC (ds m^{-1})	1.39	1.47	1.43	2.58	2.55	2.59
Total organic carbon (%)	27.2	29.8	29.4	26.9	28.1	26.5
Total C:N ratio	29:1	25:1	21:1	28:1	22:1	19:1
Total N (%)	0.91	1.17	1.38	0.96	1.27	1.39
Total P (%)	1.98	2.24	2.49	2.37	2.45	2.51
Total K (%)	2.36	2.58	3.74	2.89	3.42	3.85
Total Ca (%)	2.27	2.75	3.07	2.94	2.80	3.25
Total Mg (%)	2.18	2.74	2.94	2.60	2.57	2.99
Lignin (%)	9.54	9.12	7.30	8.72	7.67	6.69
Cellulose (%)	10.71	9.46	6.53	8.28	7.63	6.35

C Control, C_1 (GNS + *Trichoderma asperelloides* + *Eisenia fetida*), C_2 (GNS + microbial consortium), C_3 (GNS + microbial consortium + *Eisenia fetida*), C_4 (TPS + *Trichoderma asperelloides* + *Eisenia fetida*), C_5 (TPS + microbial consortium), C_6 (TPS + microbial consortium + *Eisenia fetida*)

total potassium content were noted in C_6 (3.85%) followed by C_3 (3.74%) might be attributed to the earthworms and microorganisms present in the experimental tray. During the vermicomposting process, acid producing microorganisms play an important role the solubilization of insoluble potassium in the earthworm gut (Suthar 2006). Suthar and Singh (2008) reported that vermicompost contains a high concentration of exchangeable potassium due to the enhancement of microbial activity which has also been observed from the present study.

Total calcium

Calcium is an important source for growth attributes of microbes in the composting site which increased the population of microbes and nutrition rich compost as fertilizer (Manohara et al. 2017). A higher proportion of cow dung enhanced the quality of compost thereby in this study all the treatments had a substantial amount of cow dung which promotes calcium levels. Asawalam and Onwudike (2011) stated that chemical properties of cow dung (Nitrogen 1.61%, Phosphorus 0.70%, Potassium 0.53%, Magnesium 0.91%, Calcium 2.71%, Sodium 0.50%). The calcium content in the composted GNS and TPS were increased significantly at the end of the process (Fig. 3h). The highest calcium was recorded in C_6 (3.25%) followed by C_3 (3.07%) had a higher proportion of cow dung enhanced the calcium content.

Total magnesium

The process of vermicompost significantly enhanced the magnesium content which may be due to the mineralization of organic matter. Magnesium is an important parameter for plant growth which is responsible for the production and transport of sugar for use by a plant in chloroplasts and also acts as a cofactor in photosynthesis (Ruan et al. 2011). Figure 3i shows that maximum increases of total magnesium content in the final composts were obtained from the treatments of C_6 (2.99%) followed by C_3 (2.94%), C_2 (2.74%), C_4 (2.60%), C_5 (2.57%) and C_1 (2.18) might be because of the activity of microorganisms present in the compost. Domı´nguez J Go´mez-Brando´n M (2013) and Lim et al. (2014) reported that vermicompost contains a higher amount of total magnesium content when the earthworms are incorporated into the organic substrate for the degradation process.

Lignin content

The lignin content of non-composted substrates varied from 28.5% in GNS and 37.4% in TPS shown in Table 2 which is a complex polymer composed of the most recalcitrant substance of plant biomass that binds tightly seal around the plant wall (Cesarino et al. 2012; Fang et al. 2014). According to Kerem and Hadar (1995), Khan et al. (2014) *pleurotus* species are accomplished to degrade lignin rapidly, particularly the white rot fungi *P.ostreatus* enhanced the degradation process completed within a small period and possess unique ligninolytic enzymes thereby completely degrading lignin to carbon dioxide and water (Moreira et al. 1998). In this study considerable reduction was observed in C_6 (6.69%) followed by C_3 (7.30%) and C_5 (7.67%) may be due to the presence of microorganisms (Fig. 3j). Further utilization of *Pleurotus florida* also has an important role in the consortium of microorganisms. During the lignin degradation which releases soluble substances and lignolysis byproducts thus enhance the fungal activity in the composting unit and promote the rate of degradation (Anike et al. 2016).

Cellulose content

Cellulose is a superior indicator to evaluate the degree of maturity of the compost. Raw GNS and TPS contain an enormous amount of cellulose content present in Table 2. The highest reduction was obtained in C_6 (6.35) followed by C_3 (6.53), C_5 (7.63), C_4 (8.28), C_2 (9.46) and C_1 (10.71) may be due to the activity of microorganisms present in the composting site (Fig. 3k). While during the end of the composting process cellulose and hemicellulose content completely breakdown with the help of lignolytic fungi (*Pleurotus florida*) and cellulolytic fungi (*Paecilomyces variotti*) present in the microbial consortium which enhanced the decomposition faster. The study was correlated with the finding Chen et al. (2015) lower cellulose content was observed in working with earthworms which significantly promote the degradation of cellulose.

Evaluation of compost by FT-IR spectroscopy

FT-IR analysis is one of the reliable techniques to determine the compost maturity via functional groups band nature and intensity. Generally, GNS and TPS contain certain antioxidants such as polyphenols, amino acid, gallic acid, quercetin-based compounds. These compounds presence has been observed in FT-IR analysis by their functional group's vibrational bands. In addition, the degradation process is also monitored by the disappearance of the respected bands. Figure 4a showed a comparison of FT-IR spectra of GNS, C_1, C_2 and C_3. The broad band of N–H, O–H stretching at 3371 cm^{-1} and C–H stretching vibrational band at 2970 cm^{-1} stands as strong evidence for the degradation. In addition, disappearance of O–H bending vibrational band at 1400 cm^{-1} also ensure the degradation process. C_3 got notable changes in the FT-IR analysis than C_1 and C_2 which

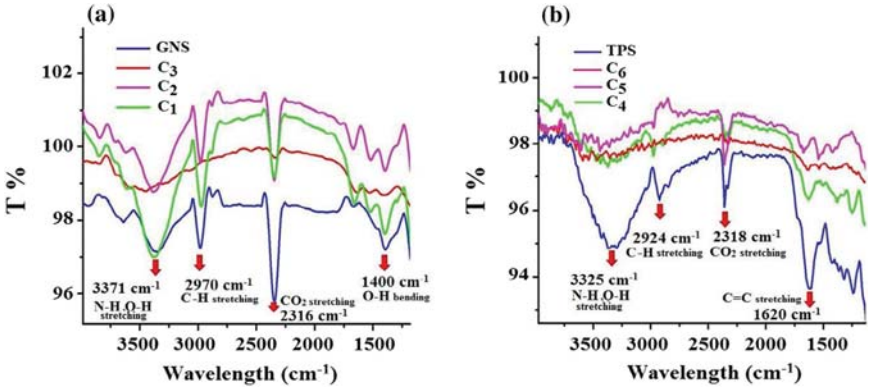

Fig. 4 FT-IR spectra of raw and composted samples. Dried compost samples were pelletized with KBr for FTIR analysis. *GNS* Groundnut shell, *TPS* Toddy palm shell, C_1 (GNS + *Trichoderma asperelloides* + *Eisenia fetida)*, C_2 (GNS + microbial consortium), C_3 (GNS + microbial consortium + *Eisenia fetida),* C_4 (TPS + *Trichoderma asperelloides* + *Eisenia fetida),* C_5 (TPS + microbial consortium) and C_6 (TPS + microbial consortium + *Eisenia fetida)*

lead to conclude the best degradation process in C_3. Figure 4b showed the comparison of TPS, C_4, C_5 and C_6. A prominent peak of TPS appears at 3325 cm^{-1} and continues to the next small peak at 2924 cm^{-1} which is attributed to (O–H, N–H) and Aromatic C–H stretching vibrational modes, respectively.

These peaks of C_6 got dramatic changes than C_4 and C_5 which ensures the level of degradation among the treatments. The stretching peak at 1620 cm^{-1} indicates the presence of (C=C) alkene has partially degraded in C_4 and C_5 where C_6 (TPS + microbial consortium + *Eisenia fetida)*-treated compost shows the best degradation. The band at 2316 cm^{-1} and 2318 cm^{-1} of GNS and TPS appeared for CO_2 stretching. In addition, a well degraded and elemental rich compost C_6 showed the same best result in physicochemical studies too.

Evaluation of compost by x-ray diffraction analysis

X-ray diffraction analysis is well known for morphological study. In an extension of FT-IR report, the degradation of compost C_3 and C_6 has been ensured by this x-ray diffraction analysis . The observed 2θ peaks at 19° and 22.5° for GNS and 18.5° and 23° for TPS were disappeared and shifted as shown in Fig. 5 which ensures the degradation of cellulose and lignin in compost C_3 and C_6, respectively.

Conclusion

Nutrient rich waste materials becoming an environmental threat was need to be stopped and utilized by the suitable recyclable methods to reach zero waste. Accordingly, GNS and TPS wastes are converted as nutrient rich organic fertilizer via an ecofriendly manner with the assistance of *Trichoderma asperelloides* and microbial consortium combination through vermicomposting using epigeic earthworm *Eisenia fetida*. Especially, the degradation rate and nutrients level of prepared composts C3 & C6 were enhanced by this combination. Plant nutrients such as N, K, P, Ca and Mg (1.39%, 3.85%, 2.51%, 3.25% and 2.99% in C_6) and (1.38%, 3.74%, 2.49%, 3.07% and 2.94% in C_3) significantly registered higher percentage accompanied by a considerable reduction of lignin, cellulose, OC and C:N ratio (6.69%, 6.35%, 26.5% and 19:1 in C_6) and (7.30%, 6.53%, 29.4% and 21:1 in C_3) than other treatments. Less than 20% of carbon nitrogen ratio indicates the quality of fertilizer thereby in this study TPS degraded with the microbial consortium and

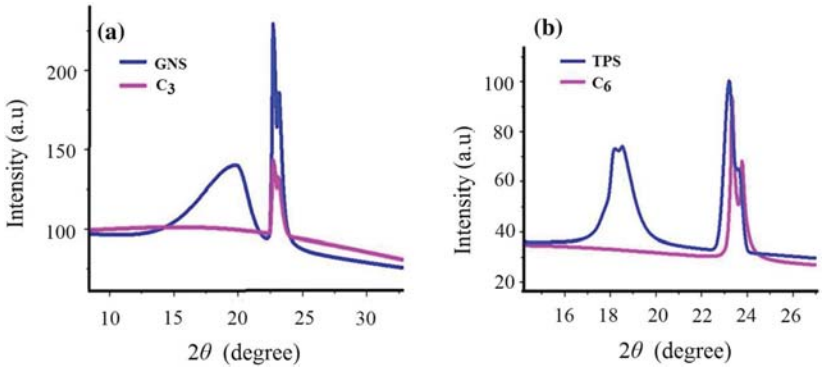

Fig. 5 XRD analysis of GNS, TPS raw and C_3, C_6 composted samples. Samples are taken for analysis in well-dried condition. *GNS* Groundnut shell, TPS Toddy palm shell, C_3 (GNS + microbial consortium + *Eisenia fetida)* and C_6 (TPS + microbial consortium + *Eisenia fetida)*

Eisenia fetida achieved a 19:1 carbon nitrogen ratio. In addition, the degradation of carboxylic acid and aliphatic groups of GNS and TPS waste was ensured by FT-IR analysis, and high degradation of cellulose was ensured by XRD analysis. All the observed results were indicating the microbial consortium and *Eisenia fetida* combination are more suitable for degradation of GNS & TPS wastes which enhance the degradation rate and the quality of fertilizers in C3 & C6 composts. Hence, the present study highlights the organic fertilizer from GNS and TPS through simple and low-cost vermicomposting technology to minimize the waste and simultaneously protect the environment. This combination has recommended to utilize the nutrients from agricultural wastes to organic fertilizer with high degradation rate and high efficiency by simple methodology.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest arising from this work.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Groundnut Shells and Toddy Palm Shells Recycling through Vermicomposting Technology and its Efficacy on Growth and Yield Attributes of Cluster Bean (*Cyamopsis Tetragonoloba* L.) Taub

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Abstract

Farming generates numerous types of agricultural wastes to the environment such as crop residues, animal waste, poultry waste etc,. those are landfilled or burning creates environmental pollutions. The aim of the study was to determining the growth and yield performance of cluster bean (*Cyamopsis tetragonoloba* L.) Taub on groundnut shells and toddy palm shells based vermicompost with *Trichoderma asperelloides*, microbial consortium and *Eisenia fetida* assistance. Pot experiments of plants were grown on six different combination of groundnut shells and toddy palm shells composts and one control treatment. During 25, 50 and 75 DAS (Days After Sowing) growth parameters and 90 DAS yield characters of cluster bean was carried out under pot culture respectively. The combined application of toddy palm shells composted with consortium of microorganisms and earthworm (T_6) achieved the maximum growth parameters such as root length (21.9cm), shoot length (84.8cm), number of leaves (46.3), number of nodules (4.6), number of flowers (24.6), number of pods (6.6), fresh weight (17.912g) and dry weight (2.684g) of plant on 75 DAS of cluster bean (*Cyamopsis tetragonoloba* L.) Taub. During the 90th day the same treatment achieved the yield characters like number of pods (8.0), length of pod (16.6cm), number of seeds/pod (10.3), yield/plant (45.384g), fresh weight (5.673g) and dry weight (1.496g) of pod compared to the control. Based on the results T_6 more suitable for growth and yield characters of cluster bean followed by T_3 respectively. The study suggested that, the organic fertilizer prepared from groundnut shells and toddy palm shells with microbial consortium and *Eisenia fetida* assistance promotes the plant development and yield attributes of *Cyamopsis tetragonoloba* (L.) Taub. simultaneously, reduce the usage of chemical fertilizers.

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Introduction

India generates approximately 350 million tonnes of agricultural waste per year. Agricultural wastes are leaf litters, crop residues, livestock, animal waste etc, those wastes are leftover product after harvesting which are landfilling or buried in the open field causing environmental pollutions. Hence, recycling is one of the best ways to reduce and use the leftover.[1] The reduction and recycling of agricultural waste in suitable and systematic ways is composting, energy production, animal fodder, mushroom production etc.,[2] In the current study, the groundnut shells and toddy palm shells recycled by composting technology. Composting is an environment friendly method converts waste into organic fertilizer with the help of animal manure (cow dung) through biological processes[3] which improving fertility of soil and crop production.[4] Animal manure is rich source of organic matter and nutrients (phosphorus and nitrogen).[5] Vermicomposting is an eco-friendly sustainable technology that involves stabilization of organic matter by dual action of microorganisms and earthworms[6] which is a peat like material used as soil conditioner, contains macro and micronutrients available in the form that are easily absorbed by plants.[7,8] Vermicompost retains water holding capacity, high porosity and humidity produced by the activity of earthworms in the composting unit which improves the plant growth, yield and soil physical and chemical parameters.[9] Vermicomposting of groundnut shells and toddy palm shells with consortium of microorganisms, *Trichoderma asperelloides* and earthworm assistance. Groundnut shells are waste material after separation of groundnut seeds and toddy palm shells are discarded material after removal of sweet jelly sockets. Both wastes are degrading very slow in the natural environmental conditions due to high level of moisture content. However, both waste material contains substantial amount of plant nutrients such as NPK.[1]

The pit composting of groundnut shells and toddy palm shells were done at Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India which is coolest method for bio-composting and low-priced. The cluster bean (*Cyamopsis tetragonoloba* L.) Taub variety MDU 1 was used as an experimental crop under pot culture studies which is an economical important and edible crop cultivated throughout India, particularly north parts.[10] Cluster bean has been considerably valued for its galactomannan content gained from the endosperm of seeds.[11] In this study, we aim to investigate the influence of six different organic fertilizer made from two different substrates on growth and yield of cluster bean respectively.

Materials and Methods

Experimental Setup

Groundnut shells was collected from groundnut field and toddy palm shells was collected from roadside vendor shops around Coimbatore located at 11.0168ºN latitude and 76.9558ºE longitude. Microorganisms, *Trichoderma asperelloides* and cluster bean seeds were collected from Tamil Nadu Agricultural University, Coimbatore. Approximately, 100ml of consortium of microorganisms such as *Streptomyces lavendulae* (MTCC-6821), *Paecilomyces variotti* (MTCC-6581), *Bacillus licheniformis* (MTCC-10498), and *Pleurotus florida* (MTCC-9194) were mixed with 10 L of water contains one kilogram of cow dung respectively. Six pits were dug in equal size measured at 50×45×60cm (length, width, depth) and named as T_1-T_6 5kg of samples (groundnut shells and toddy palm shells) transferred into respective pits along with microorganisms and cow dung in the following manner.

T_1 - Groundnut shells + *Trichoderma asperelloides* + *Eisenia fetida*
T_2 - Groundnut shell + microbial consortium
T_3 - Groundnut shells + microbial consortium + *Eisenia fetida*
T_4 - Toddy palm shells + *Trichoderma asperelloides* + *Eisenia fetida*
T_5 - Toddy palm shell + microbial consortium
T_6 - Toddy palm shells + microbial consortium + *Eisenia fetida*

After 30 days of pre-digestion process approximately 50 g of tiger worm *Eisenia fetida* was inoculated into the respective composts (T_1, T_3, T_4 and T_6) for two months and retaining moisture level around 50 percent respectively. After 90 days of complete composting process the compost was matured turn dark brown to black colour, which is used for pot culture studies. 100g of respective compost mixed

in a cement pot contains 20kg of red clay loam soil prepared having following combination and arranged in randomized block design with three replications.

C - 20kg soil
T_1 - 20kg soil + 100g (Groundnut shells + *Trichoderma asperelloides* + *Eisenia fetida*)
T_2 - 20kg soil + 100g (Groundnut shell + microbial consortium)
T_3 - 20kg soil + 100g (Groundnut shells + microbial consortium + *Eisenia fetida*)
T_4 - 20kg soil + 100g (Toddy palm shells + *Trichoderma asperelloides* + *Eisenia fetida*)
T_5 - 20kg soil + 100g (Toddy palm shell + microbial consortium)
T_6- 20kg soil + 100g (Toddy palm shells + microbial consortium + *Eisenia fetida*)

Three seeds were sowed in each pot at 3cm depth and irrigated necessary of water two days once.

Growth and Yield Characters

After germination three healthy plants were taken for analysis. On 25, 50 and 75 DAS root length, shoot length, number of leaves, flowers, nodules, fresh weight and dry weight of plant were analyzed. On 90 DAS yield parameters like number of pods, length of pod, number of seeds/pod, yield/plant, fresh and dry weight of pod were analyzed. Root length was measured from ground level to tip of the root and shoot length was measured from ground level to the shoot apex. Number of leaves, flowers, nodules, pods, seeds/pod were count manually and fresh & dry weight of plant and pods were weighted in grams by digital balance.

Statistical Analysis

The data obtained from 25, 50, 75 and 90 DAS were subjected to statistically analyzed by one way and two-way anova and based on the results inference were drawn.

Table 1: Growth parameters of (*Cyamopsis tetragonoloba* L.) Taub on 25, 50 and 75 DAS

Treatments	Root length (cm)			Shoot length (cm)		
	25 DAS	50 DAS	75 DAS	25 DAS	50 DAS	75 DAS
C	3.2	8.9	10.5	17.2	42.4	60.2
T_1	5.3	11.3	12.5	19.3	48.9	69.9
T_2	6.3	13.4	13.9	20.3	54.6	66.4
T_3	7.2	20.2	21.4	22.4	66.4	83.0
T_4	5.9	14.9	15.6	18.2	59.4	68.3
T_5	6.9	10.3	13.2	20.4	63.6	80.2
T_6	9.1	20.8	21.9	24.6	78.2	84.8
SEd		0.16441			0.20902	
Cd(p<0.05)		0.33186			0.42191	
Cd(p<0.01)		0.44361			0.56398	

Results and Discussions

Root Length (cm)

The data presented in table 1 shows that the root length and shoot length of cluster bean on 25, 50 and 75 DAS respectively. The maximum root length was observed in T_6 (9.1 cm, 20.8 cm and 21.9 cm) followed by T_3 (7.2 cm, 20.2 cm and 21.4 cm) compared to the C (3.2 cm, 8.9 cm and 10.5 cm) on 25, 50 and 75 days after sowing. This is may be due to the combined action of consortium of microorganisms and earthworms promotes maximum plant growth such as root and shoot length. The vermicompost promotes availability of macro & micronutrients and phytohormones that stimulates the plant growth.[12] In addition, the presence of PGPM in the consortium of microorganism (B. *licheniformis* and *P. variotii*) helps growth of root and shoot. These microbes are able to produce auxin which plays a vital role in elongation of root consequence plant growth.[13] Several reports have revealed that the plant inoculated with two or more organisms (microbial consortium) significantly improved the growth of plant than single source.[14,15] Likewise, the plant height significantly improved by the plant inoculated

with the dual application of recommended dose of N, P, K and microbial consortium[16] and plant growth promoting rhizobacterial strains treated *Amaranthus* crop enhanced the maximum root and shoot length compared to the control.[17]

Shoot Length (cm)

The maximum shoot length was observed in toddy palm shell composted with consortium of microorganisms and earthworm (24.6 cm, 78.2 cm and 84.8 cm) compared to the other treatments as shown in table 1. This is may be due to the obtainability of vital nutrients from consortium of microorganisms enhanced the plant growth. The supplement of macro nutrients (N, P, K) availability in T_6 promotes vigorous production of crop increase the shoot length. Particularly, P plays an important role in development of plant and multiplication of cells resulting enhanced the length of shoot.[18] The joint action of vermicompost and microbial consortium promotes maximum length of shoot. Similarly, the joint action of 75% vermicompost and 100% soil increased the growth parameters of okra. In the present study, the PGPM such as *P. variotii* and B. *licheniformis* in the consortium of microorganisms ability to produce gibberellins, responsible for the elongation of shoot.[19]

Number of leaves

On 25, 50 and 75 DAS the higher count of leaves was observed in the combined application of toddy palm shells + microbial consortium + *Eisenia fetida* (9.0, 43.3 and 46.3) followed by groundnut shells + microbial consortium + *Eisenia fetida* (8.3, 40.6 and 44.3) as given in table 2. The dual action of two or more microorganisms with vermicompost revealed the vigorous production of plant growth and also the availability of micro and macro nutrients in the vermicompost improved the growth parameters. Similarly, the mutual action of *Bacillus* sp and *Pseudomonas* sp. increased the root length, height of plant, number of leaves and yield of zea mays[20] and also vermicompost of 60% municipal solid waste enhanced the number of leaves and diameter of leaf in bhendi.[21] The application of 20% rumen blood with 80% coir pith enhanced the number of leaves of okra (*Abelmoschus esculentus*).[22]

Table 2: Growth parameters of (*Cyamopsis tetragonoloba* L.) Taub on 25, 50 and 75 DAS

Treatments	Number of leaves			Number of nodules		
	25 DAS	50 DAS	75 DAS	25 DAS	50 DAS	75 DAS
C	3.0	28.0	29.3	5.3	7.3	1.3
T_1	3.6	29.6	31.6	6.0	8.0	1.6
T_2	5.3	30.3	36.6	6.3	10.3	2.6
T_3	8.3	40.6	44.3	8.0	13.6	4.0
T_4	5.6	37.0	39.0	7.0	11.6	3.0
T_5	6.0	32.3	35.0	7.6	12.6	3.6
T_6	9.0	43.3	46.3	8.6	14.0	4.6
SEd		0.27208			0.15908	
Cd(p<0.05)		0.54921			0.32111	
Cd(p<0.01)		0.73414			0.42923	

Number of Nodules

The maximum count of root nodules presents in T_6 (8.6, 14.0 and 4.6) followed by T_3 (8.0, 13.6 and 4.0), T_5 (7.6, 12.6 and 3.6), T_4 (7.0, 11.6 and 3.0) and minimum in C (5.3, 7.3 and 1.3) on 25, 50 and 75 DAS respectively. The count of nodules reach peak on 50 DAS of plant growth which may be due the better nutrients supply enhanced the propagation and development of roots consequence better formation of nodules and fixation of nitrogen.[23] The joined action of consortium of microorganisms and earthworms promotes the highest number of nodules in both (toddy palm shell and groundnut shell) composts. Further, the application of 75%

recommended dose of fertilizer + rhizobium + pressmud increased the height of plant, number of nodules and a diameter of cluster bean under alley cropping system[15] and the combination of Brevi *bacillus* MP4 + *Pseudomonas* MP5 + Rhizobium MP7 enhanced the root nodules of *Vicia faba* plant.[24]

Number of Flowers

Higher number of flowers was observed from T_6 (toddy palm shell + microbial consortium + *Eisenia fetida*) followed by T_3 (groundnut shell + microbial consortium + *Eisenia fetida*) than C (Table 3). This might be due to the essential amount of micro and macronutrients from the consortium of microorganisms enhanced the flowering attributes of cluster bean. The recommended level of P, N, K from inorganic and organic substrate promotes maximum count of flowers.[25] The presence of PGPR in microbial consortium ability to produce auxin, gibberellins and cytokinin which plays a key role in initiation of flowering.[26]

Number of Pods

Table 3: Growth parameters of (*Cyamopsis tetragonoloba* L.) Taub on 50 and 75 DAS

Treatments	Number of flowers		Number of pods
	50 DAS	75 DAS	75 DAS
C	8.3	9.0	2.3
T_1	11.3	14.0	3.0
T_2	13.6	16.3	2.6
T_3	15.0	18.6	5.6
T_4	12.0	15.0	4.6
T_5	14.3	16.0	5.3
T_6	15.6	24.6	6.6
SEd	1.35111		0.3324
Cd(p<0.05)	2.76771		0.7130
Cd(p<0.01)	3.73404		0.9895

The maximum number of pods recorded in T_6 (6.6) followed by T_3 (5.6), T_5 (5.3), T_4 (4.6), T_1 (3.0), T_2 (2.6) and minimum in C (2.3) on 75 days after sowing which may be due to the availability of nitrogen leads to increase number of pods in cluster bean. Similarly, accumulation of nitrogen level enhanced the number of pods in mungbean.[27] In addition, the consortium of microorganisms accelerates the growth of plant and yield. The application of 100% recommended dose of fertilizer + zinc + phosphate solubilizing bacteria significantly registered maximum number of pods in *Cyamopsis tetragonoloba* (L.).[28]

Fresh Weight of the Plant (g)

On 25 DAS the maximum fresh weight was observed in T6 (4.881 g) which was gradually increased more than double fold (9.658 g and 17.912 g) on 50 and 75 DAS as shown in table 4 respectively. The plant growth promoting microorganisms present in compost enhanced the soil fertility thereby, increased the height of plant resulting increased the fresh weight. Okra inoculated with 25% urea + 75% jeewamirta enhanced the fresh weight of root and shoot.[29] In the current study, the application of microbial consortium and earthworms which have growth regulars such as gibberellins and cytokines, helps in producing more biomass of plant.[30] The isolation of rhizobium enhanced the seedling growth parameters (shoot length, root length, plant height, fresh and dry weight) of green gram[31] and different concentration of Na_2SO_4 enhanced the length and fresh weight of sesame seedling after 96 and 144 hours.[32]

Dry Weight of the Plant (g)

The maximum plant dry weight (0.894 g, 1.829 g and 2.684 g) was observed from the treatment of toddy palm shells degraded with consortium of microorganisms and earthworms assistance on 25, 50 and 75 DAS. The absorption of K+ ions and

H_2O from soil transported all parts of plant by xylem through root hairs promotes plant biomass and growth. The plant contains 70% of water content promotes fresh weight and loss of water improved the dry mass of plant. Likewise, the usage of water in plants directly related to increasing or decreasing dry weight of plant.[33]

Yield

Table 4: Fresh and dry weight of (*Cyamopsis tetragonoloba* L.) Taub on 25, 50 and 75 DAS

Treatments	Fresh weight (g)			Dry weight (g)		
	25 DAS	50 DAS	75 DAS	25 DAS	50 DAS	75 DAS
C	3.052	8.081	12.529	0.145	0.475	0.725
T1	3.296	8.984	13.021	0.255	0.845	0.986
T2	3.652	8.672	14.527	0.481	0.924	1.328
T3	4.051	9.241	17.170	0.852	1.570	2.082
T4	4.011	9.024	16.926	0.523	0.862	1.296
T5	3.958	9.052	16.098	0.686	1.224	1.841
T6	4.881	9.658	17.912	0.894	1.829	2.684
SEd		0.19268			0.17792	
Cd(p<0.05)		0.38894			0.35914	
Cd(p<0.01)		0.51991			0.48007	

The yield attributing characteristics of cluster bean like number of pods, number of seeds/pod, length of pod (cm), yield/plant (g), fresh and dry weight of plant (g) are presented in Figure 1.

The plant treated with toddy palm shells + microbial

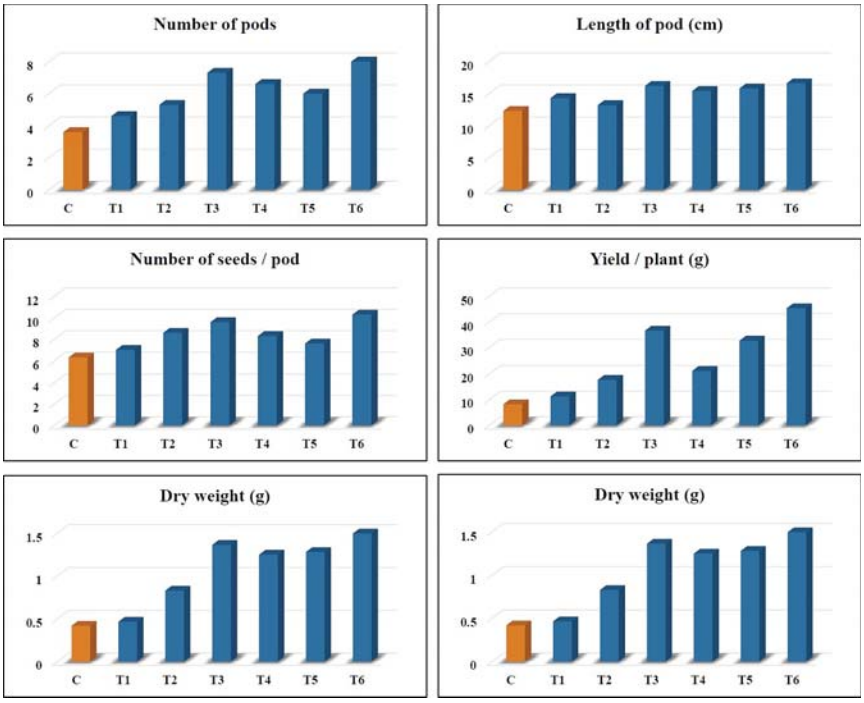

Fig. 1: Yield characters of (*Cyamopsis tetragonoloba* L.) Taub on 90 DAS

consortium + *Eisenia fetida* (8.0, 16.6 cm, 10.3, 45.384 g, 5.673 g and 1.496 g) registered maximum yield parameters of cluster bean on 90 DAS followed by T_3 (7.3, 16.2 cm, 9.6, 36.646 g, 5.020 g and 1.363 g) compared to the C (3.6, 12.3 cm, 6.3, 8.247 g, 2.291 g and 0.419 g) respectively. This might be due to better translocation of photosynthesis from leaves to pods caused higher yield in cluster bean. The joint application of organic and chemical fertilizer enhanced the yield attributing characters of cucumber.[34] The organic fertilizers like compost and vermicompost retains growth promoting substances like micro and macronutrients, bacteria, fungi, actinomycetes and vitamins.[35] The compost prepared from toddy palm shells have higher amount of essential nutrients like nitrogen, phosphorus and potassium promotes maximum yield.[1] Particularly, phosphorus is essential for initiation of flowering and fruiting. In the current study, T_6 achieved higher count of flowers promotes maximum number of pods consequence higher yield. Different combination of organic briquettes (20% rumen blood with 80% coir pith promotes maximum length of fruit, weight of fruit and fruit yield per plant in okra was reported by.[22]

Conclusions

Vermicompost with microbial consortium had positive impact on vegetative growth particularly root and shoot development promotes the growth of plant. Based on the observations, the combined application of toddy palm shells and groundnut shells degraded with consortium of microorganisms and earthworm (T_6 and T_3) is recommended for enhancement of growth characters and yield attributes of (*Cyamopsis tetragonoloba* L.) Taub. The usage of organic fertilizers enhanced the fertility of soil and higher yield simultaneously, reduce the usage of chemical fertilizers and avoids pollutions, retains water holding capacity. Hopefully this information will encourage small scale farmers to do organically and environmentally friendly ways.

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Conflict of Interest

The authors declare no conflict of interest.

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1. INTRODUCTION

Agriculture play an important role in the world economy, particularly in India as many people are dependent on agriculture. Most farmers use chemical fertilizers for the cultivation of vegetables and seasonal crops. Chemical fertilizers are used to strengthen the fertility of the soil and increase crop yield. However, it had a negative impact on soil microorganisms, biochemical and geochemical cycles. The usage of toxic synthetic fertilizers plays a vital role in agro-ecosystem which causes various harmful effects like soil degradation, genetic diversity of crop loss, soil microorganisms reduction, groundwater resource contamination and atmospheric pollution (Erana *et al*., 2019). The continuous routine usage of chemical fertilizers declines the yield of crops due to the soil acidification and loss of physico-chemical properties (Ghimire *et al*., 2017). Hence, it is necessary to stop the conventional farming system and to practice agriculture through organic in order to reduce pollution and maintain healthy environment.

Organic agriculture is a holistic production management that promotes the biological cycle of soil and biodiversity (Vijayanand *et al*., 2014) that uses fertilizers of organic origin such as compost manure, green manure, farmyard manure, biofertilizers and vermicompostwhich enrich the soil properties and promotes the crop growth and yield.Organic manures can serve as an alternative to mineral fertilizers for improving microbial biomass (Dhuller *al*., 2004) and soil structure (Dauda *et al*., 2008) which plays a direct role in plant growth as a source of all necessary micro and macro nutrients in available forms during mineralization (Chaterjeeet *al*., 2005). The use of organic fertilizer enhances soil physical, chemical and biological properties, and act as a good soil conservator by upholding its fertility, securing sustainable agricultural production and improving the quality of crop (Islami *et al*., 2011; Adhami *et al*., 2014). At the same time, agriculture faces another problem is the disposable of agricultural wastes.

The disposal of solid waste is a prominent environmental problem all over the world. The improper disposal of those wastes creates environmental pollution and vector-bornediseases spread by insects and rodents could result in health hazards to human beings.The accumulation of waste generated from industries, municipal solid waste, domestic waste and agricultural wastes in the form of liquid, slurry and solid depends on the nature of the waste.Indiagenerates 500 million tons of agricultural waste per year. Agricultural wastes are crop residues, weeds, leaf litter, straw waste and forest wastes which are disposed of by dumping or burning in the open

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