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Appendices

APPENDICES**APPENDIX 1****ESTIMATION OF LIGNIN****(GOERING AND VANSOEST, 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% H₂SO₄ 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% H₂SO₄ (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 pre weighed 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 colourless.
- ❖ Dried at 100°C for overnight.
- ❖ Weighed after cooling in a desiccator.
- ❖ ADF content was expressed in percentage i.e., $W/S \times 100$, 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 hrs with an intermittent stirring with a glass rod.
- ❖ The acid was diluted with distilled water and filtered with pre weighed 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°C 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°C 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% H₂SO₄ and followed the steps from 2 - 5.

Calculation

$$ADL (\%) = \frac{\text{Weight 72\% H}_2\text{SO}_4 \text{ washed fibre} - \text{Ash} - (\text{Test} - \text{Asbestos blank})}{\text{Weight of sample}} \times 100$$

APPENDIX 2
ESTIMATION OF CELLULOSE
(UPDEGROFF, 1969)

Principle

Cellulose undergoes acetolysis with acetic/nitric reagent forming acetylated cello dextrins which get dissolved and hydrolyzed to formed glucose molecules upon treatment with 67% H₂SO₄. 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 hrs 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 hr. 1ml of the solution was taken and diluted to 100ml. From the above diluted solution, 1ml was taken, to which 10ml 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 3

ESTIMATION OF ORGANIC CARBON

WET CHROMIC ACID OXIDATION METHOD

(WALKLEY AND BLACK, 1934)

Principle

Organic carbon present in organic matter is oxidised 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 $\text{K}_2\text{Cr}_2\text{O}_7$ 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 0.5gm of soil (passed through 0.2 mm sieve) was weighed and transferred to 500 ml conical flask. 10ml of 1N $\text{K}_2\text{Cr}_2\text{O}_7$ was added and mixed well by swirling the flask. Added 20ml 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 form

20-30 minutes. Kept the flask on asbestos sheet to avoid burning of table due to intense heat. Added 200ml of water after 30 minutes. Then added 10 ml of phosphoric acid and 1 ml of diphenylamine indicator. Titrated the solution with 0.5N 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 0.5N ferrous ammonium sulphate consumed was noted.

CALCULATION

Weight of soil taken = 0.5g

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)

Xml of $FeSO_4$ reduces 10ml 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 = $10 - (10 * Y/X)$

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

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

This is present in 0.5gm of soil

Therefore, in 100gm = $10 - (10 * Y/X) * 0.003 * 100 / 0.5$

Organic matter (surface soil) = organic carbon * 1.724

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

APPENDIX 4
ESTIMATION OF TOTAL NITROGEN
MICROKJELDHAL METHOD
(HUMPHRIES, 1956)

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: 0.5g bromocresol green and 1g of methyl red were dissolved in 100ml of 90% ethyl alcohol.
- ❖ 40% sodium hydroxide solution.
- ❖ 2% boric acid.
- ❖ Concentrated sulphuric acid (0.02 N).

Procedure

- ❖ A quantity of 0.2g of dried, sieved and homogenized sample was taken in a micro kjeldhal digestion flask (50ml capacity), to which, 12ml 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 up to 100ml 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, 10ml 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 5

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, 5g 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 o 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 HNO₃ 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 P₂O₅

(a-b) ml of 0.1619N KOH = 0.0005 x (a-b) x gm P₂O₅

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 x 100/W

Percentage of P₂O₅ 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 6

ESTIMATION OF TOTAL POTASSIUM

FLAME PHOTOMETER METHOD

(JACKSON, 1973)

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 loose an electron completely. When excited atoms

return to lower energy levels, light of characteristic wavelength is emitted. The flame photometer measures this radiation intensity which is proportional to the concentration in a solution.

Preparation

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

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 the metre reading showed zero with distilled water and 100 with 100 ppm solution 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 = $A/106 \times 250$

This was present in 50 ml of HCL extract

Therefore, in 500 ml = $A/106 \times 250 \times 500/50$ g

This was present in W gm of sample

Therefore, in 100 gm = $A/106 \times 250 \times 500/50 \times 100/W$ g

Percentage of K on moisture free basis = $A/106 \times 250 \times 500/50 \times 100/W \times 100/$
(100 – M) (M – Moisture content of sample)

APPENDIX 7

ESTIMATION OF CALCIUM AND MAGNESIUM

VERSANATE METHOD

(JACKSON, 1973)

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 neutralise the activity (red litmus turns blue) and another 5ml 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 filterate into a porcelain basin.
- ❖ Add ammonium chloride – ammonium hydroxide buffer solution drop by drop to neutralise the acidity (use red litmus paper) and 5 ml excess to maintain the pH at 10.
- ❖ Add 2 – 3 drop 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 R₂O₃ estimation = 50 ml

Volume of R₂O₃ filterate made up to = 250 ml

Volume of R₂O₃ filterate 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

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

Percentage of magnesium on moisture free basis

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

M = Moisture basis

APPENDIX 8

ESTIMATION OF PROTEIN (LOWRY *et al.*, 1951)

Principle

The blue colour developed by the reduction of the phosphomolybdic-phosphotungstic components in the Folin-Ciocalteu 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 ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 1% potassium sodium tartrate (Reagent B).
- ❖ Alkaline copper solution: 50 ml of reagent A and 1ml of reagent B were mixed prior to use (Reagent C).
- ❖ Folin-Ciocalteu reagent (Reagent D).
- ❖ Protein solution (stock standard): Weighed accurately 50mg 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 50mg 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 1ml of aliquots of the working standard were pipetted into a series of test tubes 0.1ml and 0.2ml of the sample extract in two other test tubes. The volume was made up to 1ml in all test tubes. A test tube with 1ml of water served as the blank. 5ml 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

$$100\text{gm sample} = \frac{\text{Mg of protein}}{\text{volume of test standard}} \times \text{concentration of the standard}$$

APPENDIX 9

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 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: 200mg anthrone was dissolved in 100ml of ice cold 95% H₂SO₄ and it was prepared fresh before use.
- ❖ Standard glucose: (Stock) 100mg of glucose was dissolved in 100ml water.
- ❖ Working standard – 10ml of stock solution was diluted in 100ml distilled water and stored in a refrigerator after adding a few drops of toluene.

Procedure

100mg of the sample (leaf) was taken in a boiling tube with 5ml 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 100ml and centrifuged. The supernatant was collected and 0.5 and 1ml 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 1ml and '0' served as blank. The volume was made up to 1ml in all the test tubes including the sample test tubes by adding distilled water. Then, 4ml 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 630nm. A standard graph was drawn by plotting concentration of the standard on the x 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 10

ESTIMATION OF CHLOROPHYLL

(ARNON, 1949)

Principle

Chlorophyll was extracted in 80% acetone. The absorption at 663 nm, 645 nm and 652nm 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 1g of finely cut and well mixed representative leaf sample. It was ground to a fine pulp with the addition of 20ml of 80% acetone with a mortar and pestle and was centrifuged as 5,000 rpm for 5 minutes. The supernatant was transferred to a 100ml volumetric flask. The residue was ground with 20ml 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 100ml 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.

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

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

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

Were,

A = absorbance of specific wavelengths

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

W = fresh weight of tissue extract

APPENDIX 11

ESTIMATION OF AVAILABLE NITROGEN IN SOIL

ALKALINE PERMANGANATE METHOD

(SUBBIAH AND ASIJA, 1956)

Principle

A known weight of soil is mixed with excess of alkaline permanganate and distilled organic matter present in soil is oxidised by the nascent oxygen liberated by KMnO₄ 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 H₂SO₄.

Reagents

- ❖ 0.32% KMnO₄ solution (3.2 gm of KMnO₄ dissolved in one litre of distilled water).
- ❖ 2.5% NaOH solution (25 gm of NaOH dissolved in one litre of distilled water).
- ❖ 2% boric acid (20 gm of boric acid dissolved in one litre of distilled water).
- ❖ N/50 H₂SO₄ (30 ml of Conc. H₂SO₄ is diluted to one litre with distilled water and standardized by titration with N/10 Na₂CO₃. This gives N/10 H₂SO₄. From this N/50 H₂SO₄ 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 gm 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 $\text{N}/50 \text{ H}_2\text{SO}_4$.

Calculation

Weight of the soil taken = 20g

Volume of $\text{N}/50 \text{ H}_2\text{SO}_4$ = X ml (titre value)

1 ml of $\text{N}/10 \text{ H}_2\text{SO}_4$ = 0.0014 g N

Therefore 1 ml of $\text{N}/50 \text{ H}_2\text{SO}_4$ = 0.00028 g N

X ml of $\text{N}/50 \text{ H}_2\text{SO}_4$ = 0.00028 * X g N

This is present in 20 g of soil

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

APPENDIX 12

ESTIMATION OF AVAILABLE PHOSPHORUS IN SOIL

CALORIMETRY METHOD

(BRAY 1 METHOD – JACKSON, 1973)

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 is estimated calorimetrically as available phosphorus.

REAGENTS

- ❖ NH₄F solution (1N): 37g of NH₄F 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 NH₄F and 0.02 N HCL]: 15 ml of 1N NH₄F and 25 ml of 0.5N HCL are mixed and the volume was up to 500 ml with distilled water.
- ❖ Ascorbic acid.

PROCEDURE

Weighed 5g of 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 = 5g

Volume of NaHCO₃ = 50 ml

Volume of extractant solution used for

Phosphorus estimation (aliquoreo) = 5 ml

Calorimeter reading = T

Concentration of phosphorus read from

standard graph for the reading T = X ppm

= X mg/ml

= X/106 gm/ml

Therefore in 25 ml of solution = X/106*25g

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

Therefore, available P₂O₅ in kg/ha = X *25 *50 *2 *10⁶10⁶ *5 *5

APPENDIX 13

ESTIMATION OF AVAILABLE POTASSIUM IN SOIL

FLAME PHOTOMETRY METHOD

(STANDFORD AND ENGLISH, 1949)

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 5g of soil into a polythene shaking bottle. Added 25 ml of 1 N ammonium acetate and contents shaken in a mechanical reciprocating shaker for 5 minutes. Contents filtered through whatman No. 40 filter paper. Filterates 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/10⁶ gm/ml

Therefore in 25 ml solution = X/10⁶*25g

This is present in 5gm of soil

Therefore, available K in soil in kg/ha = X/10⁶*25*2*10⁶/5

APPENDIX 14

DPPH RADICAL SCAVENGING ACTIVITY

(MENSOR *et al.*, 2001)

Principle

DPPH radical reacts with an antioxidant compound that can donate hydrogen, and gets reduced. DPPH, when acted upon by an antioxidant, is converted into diphenyl picryl hydrazine. This can be identified by the conversion of purple to light yellow colour.

Reagents

1. DPPH - 2, 2-diphenyl-2-picryl hydrazyl hydrate (0.3mM in methanol)
2. Methanol

Procedure

The extracts (20µl) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the extracts, served as the positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer. The radical scavenging activity was calculated as follows

$$\text{Scavenging activity \%} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

APPENDIX 15

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

(RUCH *et al.*, 1989)

Principle

The UV absorption of hydrogen peroxide can be easily measured at 230 nm. On scavenging of hydrogen peroxide by the plant extract, the absorption decreases at this wavelength. This property is utilized to quantify their H₂O₂ scavenging ability.

Reagents

1. Phosphate buffer (0.1M, pH 7.4)
2. H₂O₂ (40mM) in phosphate buffer

Procedure

A solution of H₂O₂ (40mM) was prepared in phosphate buffer. Plant extracts at the concentration of 5µl were added to H₂O₂ solution (0.6ml) and the final volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer. A blank solution containing phosphate buffer, without H₂O₂ was prepared. The extent of H₂O₂ scavenging of the plant extracts was calculated as

$$\% \text{ scavenging of hydrogen peroxide} = \frac{(A_0 - A_1) \times 100}{A_0}$$

A₀ - Absorbance of control;

A₁ - Absorbance in the presence of plant extracts

APPENDIX 16

NITRIC OXIDE RADICAL SCAVENGING ACTIVITY

(GREEN *et al.*, 1982)

Principle

At physiological pH, sodium nitroprusside generates nitric oxide which interacts with O₂ to produce nitrite ions, which is measured at 546 nm.

Reagent

1. Sodium nitroprusside (100 mM)
2. pH buffers saline (PBS) pH 7.4
3. Griess reagents

Procedure

Sodium nitroprusside (2 ml), phosphate buffered saline (0.5ml) and plant extract (0.5µl) were mixed and incubated at 25°C for 30 minutes. Griess reagent (0.5 ml) was added and allowed to stand for another 30 minutes. The pink colour chromophore was developed and the absorbance was read at 546 nm.

APPENDIX 17
REDUCING POWER ASSAY
(OYAIZU, 1986)

Principle

Substances, which have reduction potential, react with potassium ferricyanide (Fe_3^+) to form potassium ferrocyanide (Fe_2^+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Potassium ferricyanide + Ferric chloride \longrightarrow Potassium ferrocyanide + Ferrous chloride

Reagents

1. Potassium ferricyanide (1%)
2. Phosphate buffer (0.2 M, pH 6.6),
3. Trichloro acetic acid (10%)
4. Ferric chloride (0.1%)
5. Ascorbic acid (1%)

Procedure

0.5 ml of the plant extracts were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50°C in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm.

APPENDIX 18
ANTIBACTERIAL ACTIVITY (BAUER *et al.*, 1966)

Procedure**Inoculum Preparation****Growth Method**

The growth method is performed as follows

1. At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as Nutrient broth.

2. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity (Usually 2 to 6 hours).
3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity. This results in a suspension containing approximately 1 to 2 x 10⁸ CFU/ml for *E. coli* and *Staphylococcus aureus*.

Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
2. The dried surface of a Nutrient agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
3. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.
4. The media was punctured by making a well of 6 mm in diameter and filled with 20µl of each sample. Further the Petri plate were placed inversely for complete diffusion and inhibition zone were examined by measuring the diameter (mm) formed around the well after 24 hrs. incubation at 37°C. The zones were measured by using standard (Hi-Media) scale.

Annexures



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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2023-24/Tech ~ 352

दिनांक / Date: 13th April 2023

पादप प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen given by you for authentication is identified as
***Allium cepa* L. - AMARYLLIDACEAE.**

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.


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

सेवा में / To

Ms. K. GNANAMANI
Ph.D. Research Scholar
Department of Botany
Avinashilingam Institute for Home Science and
Higher Education for Women
COIMBATORE - 641 043



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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2022/Tech /570

दिनांक/Date: 10. 11. 2022

पादप प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen brought by you for authentication is identified as
Solanum nigrum L. - SOLANACEAE.

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/5/23/2022/Tech /569

दिनांक/Date: 10. 11. 2022

पादप प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen brought by you for authentication is identified as
***Solanum lycopersicum L.* - SOLANACEAE.**

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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दिनांक / Date: 10. 11. 2022

पादप प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen brought by you for authentication is identified as
***Solanum melongena* L. - SOLANACEAE.**

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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	Effect of composted sugarcane trash and sugarcane bagasse on the growth and yield of tomato (<i>Solanum nigrum</i> L.)	Agricultural science digest	Vol-(43) No-(3) 2023	Indexed in Scopus
2	Efficient utilization of agro-industrial waste through vermicomposting and its impact on growth and yield of brinjal (<i>Solanum melongena</i> L.)	Agricultural science digest	DOI NO- 10.18805/ E .D- 5775. 2023	Indexed in Scopus

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

Scholar : *K. Gnanamani*

Supervisor : *A. vijayalakshmi*

Checked By: *A. Vijayalakshmi*
6/10/23
HoD/Dean of Respective School

The details of publication of Mrs. Guanamani, K (17PHBOF003) are as follows:

1. Agricultural Science Digest - is indexed and active in Scopus from 2019 to present. She published her article in Vol. 43, No. 3, June 2023 Pg. 269-273 and
2. She got acceptance and DOI number from the same journal

J. J. BIL
05.10.23.

I have checked in the Scopus database, the articles are published in original "Agricultural Science Digest" journal. The accepted article will be published in December 2023 issue. The mail is received from the publisher is attached herewith for your reference.

J. J. BIL
11.10.23.

Publications



Effect of Composted Sugarcane Trash and Bagasse on the Growth and Yield of Tomato (*Solanum lycopersicum* L.)

K. Gnanamani¹, A. Vijayalakshmi¹

10.18805/ag.D-5632

ABSTRACT

Background: Organic farming proves many advantages for recycling regenerates the waste matter into wealth and can wipe out the use of chemical fertilizers and pesticides. Sustainable agriculture can be ensured in future with the help of organic farming which includes various processes of biological origin such as compost and vermicompost.

Methods: The study was conducted at Alanthurai, Coimbatore, Tamil Nadu. Agro-industrial waste of sugarcane trash and bagasse were collected and were used for biocomposting process using *Pleurotus florida*, *Trichoderma asperelloides*, microbial consortium and *Eudrilus eugeniae*. Six different treatments were incorporated in the present study. Vegetative parameters such as shoot length, root length, number of leaves, fresh weight and dry weight at different stages (30, 60 and 90 DAS), number of flowers and number of branches on 60 and 90 DAS. On 90th day yield characters like number of fruits, diameter of fruits, single fruit weight, fruit yield per plant and fruit yield per plot were analyzed.

Result: A significant increase in shoot length, root length, number of leaves, fresh weight and dry weight was observed in T₃ - C₃ (Predecomposed sugarcane trash + *Trichoderma asperelloides* and Microbial consortium 5 t/h) treatment, followed by T₁ - C₁ (Predecomposed sugarcane trash *Pleurotus florida* and earthworm (*Eudrilus eugeniae*) 5 t/h) treatment on 30, 60 and 90 DAS. A significant increase in number of flowers and number of branches was observed in T₃ treatment followed by other treatment on 60 and 90 DAS. The maximum amount in number of fruits, diameter of fruit, single fruit weight, fruit yield per plant and fruit yield per plot were noted in T₃ treatment, followed by T₁ treatment on 90 DAS and minimum amount were reported in control (soil). The results of the study clearly indicated that treatment T₃ significantly increased the vegetative parameters and yield characters in tomato.

Key words: Bagasse, DAS, *Eudrilus eugeniae*, *Pleurotus florida*, Sugarcane trash, *Trichoderma asperelloides*.

INTRODUCTION

Organic farming is an important factor in the successful cultivation of healthy plants. This method of farming can be explored as an eco-friendly and sustainable waste management approach. Organic farming consists of environmental friendly raw material that can be returned to soils as biofertilizers. On an average a hectare of sugarcane generates about 10 tons of trash. Trash contains 28.6% organic carbon, 0.35 to 0.42% nitrogen, 0.04 to 0.15% phosphorous and 0.50 to 0.42% potassium. The sugarcane trash incorporation in the soil influences physical, chemical and biological properties of the soil (Shree Harsha Kumar *et al.*, 2018). Sugarcane trash incorporation reduces the bulk density of the soil and there is an increase in infiltration rate and decrease in penetration resistance. Sugarcane trash can be easily composted by using the fungi like *Trichurus*, *Aspergillus*, *Penicillium* and *Trichoderma*. Bagasse is a lignocellulosic waste from sugar mills and agricultural processing. The plants grown on sugarcane bagasse yielded a 22% increase in root length, 20% increase in plant height and 63% increase in the number of roots. Bagasse can be used as raw material organic fertilizer and recycled in agriculture as organic fertilizer product. Bagasse also has high nutrient content that is beneficial for plant growth. Bagasse products are biodegradable and compostable. *Solanum lycopersicum* is an important vegetable crop that belongs to the family solanaceae. The species originated in

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Submitted: 05-07-2022 **Accepted:** 15-11-2022 **Online:** 02-12-2022

western South America. Root decoction is ingested for relief from tooth pain. Tomatoes are rich in lycopene, a substance with beneficial effects on the heart and prostate. It is also used for rheumatism and headaches. The main objectives of the present investigation was to evaluate the impact of sugarcane trash and bagasse on growth and yield parameters in tomato (*Solanum lycopersicum* L.).

MATERIALS AND METHODS

The present study was conducted from July to September 2019-2020 at Alanthurai (10.9536 N.7885 E) Coimbatore, Tamil Nadu. The method adopted for decomposition of agro

waste sugarcane trash and bagasse was pit composting and subjected to field experiment using randomized block design with three replications.

Collection of agro-industrial waste

The agro industrial waste of sugarcane trash and sugarcane bagasse was collected from in and around Coimbatore. It was chopped into small pieces, sun dried and preserved for further study. The collected raw samples were used for the pre-decomposition with the incorporation of *Pleurotus florida*, *Trichoderma asperelloides*, *Eudrilus eugeniae* and microbial consortium. The process of composting was conducted in 1.5 feet length and 4 square feet width compost pit. It was filled by sugarcane trash and sugarcane bagasse waste. It was allowed to decompose for 30 days. This work was done from February to April 2019.

Field culture experiment with the treatments

The field culture experiment was conducted with tomato (*Solanum lycopersicum* L.) in Alanthurai, Coimbatore. The compost was mixed thoroughly and applied to the field. Viable seeds were selected and they were sown in the field with three replications. As per recommendation of Tamil Nadu Agricultural University, Coimbatore, plant protection measures and other cultural practices were followed:

C: Control.

T₁: C₁ (Predecomposed Sugarcane trash, *Pleurotus florida* and earthworm (*Eudrilus eugeniae*) 5 t/h).

T₂: C₂ (Predecomposed Sugarcane trash, *Trichoderma asperelloides* and earthworm (*Eudrilus eugeniae*) 5 t/h).

T₃: C₃ (Predecomposed Sugarcane trash, *Trichoderma asperelloides* and Microbial consortium 5 t/h).

T₄: C₄ (Predecomposed Sugarcane bagasse, *Pleurotus florida* and earthworm [*Eudrilus eugeniae*] 5 t/h).

T₅: C₅ (Predecomposed Sugarcane bagasse, *Trichoderma asperelloides* and earthworm (*Eudrilus eugeniae*) 5 t/h).

T₆: C₆ (Predecomposed Sugarcane bagasse, *Trichoderma asperelloides* and Microbial consortium 5 t/h).

Statistical analysis

The data obtained from various observations on 30, 60 and 90 DAS were analyzed statistically using One-way and Two-way ANOVA.

RESULTS AND DISCUSSION

Vegetative parameters

Among all treatments T₃- C₃ (Predecomposed Sugarcane trash *Trichoderma asperelloides* and Microbial consortium 5 t/h) treatment registered maximum shoot length (57.1, 71.6 and 99.2 cm), root length (14.17, 22.77 and 44.20 cm), number of leaves (37.00, 81.00 and 110.33), on 30, 60 and 90 DAS, followed by T₁- C₁ (Predecomposed sugarcane trash *Pleurotus florida* and earthworm (*Eudrilus eugeniae*) 5 t/h) (50.3, 68.3 and 95.4 cm), (12.60, 20.73 and 41.73 cm), (30.67, 75.00 and 106.00), on 30, 60 and 90 DAS over the control (31.5, 47.0 and 78.3 cm), (6.40, 10.27 and 27.27 cm), (18.33, 68.00 and 90.00), on 30, 60 and 90 as shown in Table 1.

Fresh weight and dry weight

A significant increase in fresh weight (26.60, 44.93 and 52.23 g) and dry weight (5.83, 6.73 and 7.93 g) on 30, 60 and 90 DAS was observed in T₃- C₃ (Predecomposed sugarcane trash *Trichoderma asperelloides* and Microbial consortium 5t/h) treatment followed by T₁- C₁ (Predecomposed Sugarcane trash *Pleurotus florida* and earthworm (*Eudrilus eugeniae*) 5 t/h) (24.50, 43.00 and 48.67 g) and (5.53, 6.17 and 7.47 g) over the control (14.83, 23.63 and 33.83 g) and (2.53, 3.57 and 4.33 g) on 30, 60 and 90 DAS as shown in Table 2.

Number of flowers and number of branches

The results as presented in Table 3, the number of flowers and number of branches was found to be maximum in T₃- C₃ (Predecomposed Sugarcane trash *Trichoderma asperelloides* and Microbial consortium 5 t/h) treatment (34.67 and 44.67) and (16.00 and 22.00) followed by T₁- C₁ (Predecomposed Sugarcane trash *Pleurotus florida* and earthworm (*Eudrilus eugeniae*) 5 t/h) treatment of (32.00 and 42.00) and (13.00 and 20.33) when compared to the control (19.67 and 24.00) and (5.67 and 8.00) on 60 and 90 DAS.

Similar work was reported by Dhanalakshmi *et al.* (2014) that the application of vermicompost increases the shoot length (13.03, 11.53, 13.10 and 10.90), number of branches (8.00, 15.33, 16.67 and 19.47) in vegetable crops of okra, brinjal, tomato and chilli. The present study supported by

Table 1: Effect of composted sugarcane trash and bagasse on the shoot length, root length and number of leaves on *Solanum lycopersicum* L.

Treatment	Shoot length (cm)			Root length (cm)			Number of leaves		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
C	31.5	47.0	78.3	6.40	10.27	27.27	18.33	68.00	90.00
T ₁	50.3	68.3	95.4	12.60	20.73	41.73	30.67	75.00	106.00
T ₂	36.3	34.4	82.8	7.33	12.60	31.00	20.67	78.67	91.67
T ₃	57.1	71.6	99.2	14.17	22.77	44.20	37.00	81.00	110.33
T ₄	41.5	62.3	91.5	10.80	18.70	39.33	28.67	72.00	103.00
T ₅	36.9	59.8	88.6	9.03	16.83	35.73	26.00	68.33	98.33
T ₆	35.1	50.4	80.4	8.10	14.73	34.20	22.67	64.67	94.67
SED		0.36697			3.11812			3.02896	
Cd (p<0.05)		0.74074			6.29409			6.11412	

DAS- Days after sowing.

Table 2: Effect of composted sugarcane trash and bagasse on the fresh weight and dry weight of *Solanum lycopersicum* L.

Treatment	Fresh weight (g)			Dry weight (g)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
C	14.83	23.63	33.83	2.53	3.57	4.33
T ₁	24.50	43.00	48.67	5.53	6.17	7.47
T ₂	17.63	29.43	38.50	3.13	4.43	5.23
T ₃	26.60	44.93	52.23	5.83	6.73	7.93
T ₄	22.43	39.03	44.67	4.97	5.87	6.90
T ₅	19.63	35.80	42.13	4.23	5.40	6.10
T ₆	16.43	32.30	40.87	3.63	4.73	5.43
SED		2.95570			0.43680	
Cd (p<0.05)		5.96625			0.88170	

DAS- Days after sowing.

Table 3: Effect of composted sugarcane trash and bagasse on the number of flowers and number of branches of *Solanum lycopersicum* L.

Treatment	Number of flowers		Number of branches	
	60 DAS	90 DAS	60 DAS	90 DAS
C	19.67	24.00	5.67	8.00
T ₁	32.00	42.00	13.00	20.33
T ₂	23.00	26.67	6.67	11.67
T ₃	34.67	44.67	16.00	22.00
T ₄	28.67	38.67	11.33	18.33
T ₅	27.00	35.00	10.00	16.00
T ₆	26.00	31.00	8.00	14.00
SED	2.82843		2.65772	
Cd (p<0.05)	5.79393		5.44424	

DAS- Days after sowing.

Mahmud *et al.* (2020) who confirmed that the application of vermicompost showed maximum number of leaves (51) in pineapple (*Ananas comosus* var. MD2). The results coincides with Silpa and Vijayalakshmi (2022) who confirmed that the application of biocomposted cocoa shell and jack fruit peel waste increased the shoot length (175.83 cm), root length (39.23 cm) and number of leaves (37.50) in *Vigna unguiculata* (L.) Walp. The present study was supported by Raihing and Vijayalakshmi (2022) reported that the application of vermicompost increased the shoot length, root length, fresh weight and dry weight in black gram (*Vigna mungo* L.).

The present study is in agreement with Manimegala and Gunasekaran (2020) who reported that the application of vermicompost and NPK fertilizer increased the number of leaves (72.54), number of branches (16.50) and number of flowers (51.83) in egg plant (*Solanum melongena* L.). The results was on par with Priya and Santhi (2014) who confirmed that the application of vermicompost showed maximum shoot length (29.88) and root length (10.0) in *Solanum nigrum*. Similar work was reported by Sakthivigneswari and Vijayalakshmi (2016) who reported that the application of biocompost increase the shoot length (90.47), root length (60.10), number of leaves (180.33),

number of flowers (28.67), fresh weight (55.47) and dry weight (6.17) in *Solanum nigrum* L.

Similar work was reported by Sumathi *et al.* (2014) who observed that the application of vermicomposts increased the number of branches (6.120) in *Abelmoschous esculentus*. The present study is in correlation with Kavitha *et al.* (2013) who confirmed that the combined application of biofertilizer, chemical fertilizer and vermicompost increase in shoot length (13.13), root length (8.25), number of leaves (30.02), fresh weight (1.76) and dry weight (0.25) 40th days of growth in *Amranthus tristis*. The results coincides with Senthilkumar and Sivagurunathan (2012) that the application of bacterial biofertilizers increases the shoot length (30.0), root length (14.6) and number of leaves (9.6) (23.4), (7.1) and (8.8) in cowpea (*Vigna siensis* Edhl) and green gram (*Phaseolus radiata* L.).

Yield parameters

Among all treatments T₃ - C₃ (Predecomposed Sugarcane trash *Trichoderma asperelloids* and Microbial consortium 5 t/h) treatment registered maximum number of fruits/plant (47.7), diameter of fruit (7.3 cm), single fruit weight (79.07 g), fruit yield per plant (5.82 kg) and fruit yield per plot (39.66 kg) on 90 DAS, followed by T₁ - C₁ (Predecomposed sugarcane trash *Pleurotus florida* and earthworm [*Eudrilus eugeniae*] 5 t/h) (33.7, 6.3 cm, 76.57 g, 5.68 kg and 38.3 kg) on 90 DAS over the control (19.0, 3.5 cm, 56.53 g, 2.96 kg and 28.96 kg) on 90 DAS as shown in the Table 4.

The present study was correlated with the findings of Eswaran and Mariselvi (2016) who reported that the application of organic manure and vermicompost increased the number of fruits (19.43) and (22.38) in tomato (*Lycopersicum esculentum*). The present findings was supported by Mullaimaran and Haripriya (2016) who confirmed that the application of organic manures increased the single fruit weight per plant (54.42) and fruit yield per plot (48.35) in tomato. The present study is correlated with Saraswathy and Prabhakaran (2014) who observed that the application of vermicompost increased the number of fruits per plant, fruit weight, fruit weight per plant and fruit yield per plant in tomato (*Lycopersicum esculentum* Mill.).

Table 4: Effect of composted sugarcane trash and bagasse on the yield parameters of *Solanum lycopersicum* L.

Treatment	Number of fruits	Diameter of fruit (cm)	Single fruit weight (g)	Fruit yield per plant (kg)	Fruit yield per plot (kg)
	90 DAS	90 DAS	90 DAS	90 DAS	90 DAS
C	19.0	3.5	56.53	2.96	28.96
T ₁	33.7	6.3	76.57	5.68	38.37
T ₂	26.0	4.3	65.03	4.65	32.93
T ₃	47.7	7.3	79.07	5.82	39.66
T ₄	32.0	5.3	71.40	5.35	36.56
T ₅	28.0	5.2	67.80	4.86	34.88
T ₆	24.3	4.2	61.87	3.96	30.76
SEd	1.6330	0.0992	2.5165	0.0498	0.0382
CD (p<0.05)	3.5028	0.2128	5.3980	0.1068	0.0820

DAS- Days after sowing.

Similar work was reported by Singh *et al.* (2013) who reported that the application of vermicompost increased fruit weight (92.9 g) and fruit yield per plant (4.013 kg) in tomato (*Solanum lycopersicum* L.). The present finding was supported by Adhikary *et al.* (2016) who reported that the application of manures and fertilizers increased the number of fruits per plant (37.61), fruit diameter (5.33 cm), weight of individual fruit (75.14g) and fruit yield per plant (30.03 kg) in tomato.

The present study was in correlation with the findings of Palia *et al.* (2021) who reported that the application of organic and inorganic fertilizers increase the diameter of fruit (29.18 cm) in brinjal (*Solanum melongena* L.). The results was on par with Haghghi *et al.* (2016) who confirmed that the application of municipal solid waste compost, peat, perlite and vermicompost increased fruit weight (80.36 g) in tomato (*Lycopersicon esculentum* L.).

CONCLUSION

The present research is to brighten the possibilities of using sugarcane trash and bagasse waste in enhancing the crop productivity. Agro industrial waste can be recycled and used as a cheaper source of organic nutrients. Organic manures improve the soil fertility and biological properties of the soil. From the results, it can be concluded that the application of the treatment [T₃ - C₃ (Predigested sugarcane trash *Trichoderma asperelloids* and Microbial consortium 5t/h)] showed maximum on the yield parameters of Tomato (*Solanum lycopersicum* L.).

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Conflict of interest: None.

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Efficient Utilization of Agro-industrial Waste through Vermicomposting and its Impact on Growth and Yield of Brinjal (*Solanum melongena* L.)

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ABSTRACT

Background: Application of vermicompost produced from diverse organic wastes could be one of the most economical and attractive methods of solving waste disposal problem and increasing the nutrient contents of soil simultaneously. Vermicompost is used as an organic fertilizer, the effects of vermicompost obtained from different organic wastes on plant growth and yield.

Methods: The vermicomposting of sugarcane trash and bagasse waste was carried out during (January-March) and by using the vermicompost a field culture experiment was conducted in brinjal for three months from April-June, 2019. There are 6 treatments namely T₁-T₆ and control. Vegetative parameters such as shoot length, root length, number of leaves, fresh weight and dry weight at different stages (30, 60 and 90 DAS), number of flowers and number of branches on 60 and 90 DAS. On 90th day yield characters like number of fruits, diameter of fruits, fruit length, single fruit weight, fruit yield per plant and fruit yield per plot were analyzed.

Result: A significant increase in shoot length (32.9, 55.3 and 98.5), root length (17.85, 25.10 and 33.30), number of leaves (21.33, 50.00 and 77.00), fresh weight (31.10, 48.30 and 55.55) and dry weight (7.10, 7.95 and 9.30) was observed in T₅-C₅ (Predecomposed Sugarcane bagasse, *Trichoderma asperelloids* and earthworm (*Eudrilus eugeniae*) 5 t/h) treatment, followed by T₂ - C₂ (Predecomposed Sugarcane trash, *Trichoderma asperelloids* and earthworm (*Eudrilus eugeniae*) 5 t/h) treatment on 30, 60 and 90 DAS. A significant increase in number of flowers (27.50 and 55.50) and number of branches (22.50 and 27.50) was observed in T₅ treatment followed by other treatments on 60 and 90 DAS. The maximum amount in number of fruits (22.00), fruit length (15.70), single fruit weight (93.15), fruit yield per plant (10.35) and fruit yield per plot (37.25) were noted in T₅ treatment, followed by T₂ treatment on 90 DAS and minimum amount were reported in control (soil). The results of the study clearly indicated that treatment T₅ significantly increased the vegetative parameters and yield characters in brinjal.

Key words: Bagasse, Brinjal, Control, DAS, *Eudrilus eugeniae*, Sugarcane trash, *Trichoderma asperelloids*.

INTRODUCTION

Organic manures for growing crops are a composition of waste materials. Composting of organic waste offers solution to large amounts of waste worldwide. Vermicomposting is a type of organic farming by which earthworms breakdown organic waste materials, stimulate microbial activity and at the same time, increase the rate of mineralization of the soil. The technique of organic farming plays a role in cultivation of high value of vegetable crops. Application of vermicompost to field soils have also been reported to increase crop growth and yields. Sugarcane is one of the important cash crops in India and plays essential role in both agricultural and industrial economy of the country. India approximately 6.5 million tonnes of sugar cane trash are being produced every year and most of the residues are usually burnt in the field due to lack of proper composting techniques. Besides the loss of organic matter and plant nutrients, burning of crop residues also causes atmospheric pollution due to the emission of toxic gases methane, carbon dioxide that poses threat to human and ecosystem. *In situ* composting of cane trash can be a good alternate to mitigate these problem Viji and Neelanarayanan (2016). Bagasse is composed largely of cellulose, pentose and lignin. It is made up of 45-55% of cellulose, 20-25% of hemicelluloses and 18-24% lignin Surya

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et al. (2015). Brinjal popularly known as egg plant belongs to family solanaceae, one of most common vegetable crops grown in India. Brinjal is staple vegetable in almost all tropical countries in the world and utilized by both poor and rich. Its nutritive value varies among varieties. It contains vitamin A and B. The brinjal fruit contains moisture 92.7 g, protein 1.4 g, phosphorous 47 mg, iron 0.9 mg, vitamin C 10 mg, riboflavin 0.11 mg, thiamine 0.04 mg more while brinjal may have the medicinal property. White brinjal which is used for Diabetics

patients (Palia *et al.*, 2021). The main objectives of the present investigation was to evaluate the impact of sugarcane trash and bagasse on growth and yield parameters in brinjal (*Solanum melongena* L.).

MATERIALS AND METHODS

The present study was conducted from January to March 2019 at Alanthurai (10.9536 N, 76.7885 E), Coimbatore, Tamil Nadu, India. The method adopted for decomposition of agro waste sugarcane trash and bagasse was pit composting and subjected to field experiment using randomized block design with three replications.

Collection of agro-industrial waste

The agro industrial waste of sugarcane trash and sugarcane bagasse was collected from in and around Coimbatore. It was chopped into small pieces, sun dried and stored in bags.

Compost pit preparation

The process of composting was conducted in 1.5 feet length and 4 square feet width compost pit. It was filled by sugarcane trash and sugarcane bagasse waste. It was allowed to decompost for 30 days.

Sugarcane trash compost

Compost 2 kg of sundried sugarcane trash agro-waste was transferred to C₁ pit, spread with 20 g of *Pleurotus florida* spawn was sandwiched uniformly. This process was repeated till the heap reaches a height of above 1 meter. The moisture content was maintained by sprinkling water with regular interval. Turning the agro-waste was manually done every week using composting process. Vermicomposting process was adopted by adding 10 to 15 earthworms after 30 days.

Compost 2 pit was filled with 2 kg of sugarcane trash waste along with 20 g of *Trichoderma asperelloids* was added. This process was repeated till the heap reached a height of above one meter. Manual turning was done every week during composting period to accelerate the decomposition process. After 30 days of composting, vermicomposting process was adopted.

Compost 3 pit was filled by sundried sugarcane trash waste. 20 g of *Trichoderma asperelloids* and 25 ml of microbial consortium was added. The process was repeated till the heap reached a height of above 1 meter. The pit moisture content was maintained to 60-70% by sprinkling of water with regular interval. Turning of compost was manually done every week.

Sugarcane bagasse compost

Same technique was repeated in place of sugarcane trash (C1, C2 and C3) sugarcane bagasse was used in the following composting pits compost 4 (C4) compost 5 (C5) and compost 6 (C6) respectively.

Field culture experiment with the treatments

The field culture experiment was conducted with brinjal (*Solanum melongena* L.) in Alanthurai, Coimbatore, Tamil Nadu.

The compost was mixed thoroughly and applied to the field. Viable seeds were selected and they were sown in the field with three replications. As per recommendation of Tamil Nadu Agricultural University, Coimbatore, plant protection measures and other cultural practices were followed.

C	Control
T ₁	C ₁ [Predecomposed Sugarcane trash, <i>Pleurotus florida</i> and earthworm (<i>Eudrilus eugeniae</i>) 5 t/h]
T ₂	C ₂ [Predecomposed Sugarcane trash, <i>Trichoderma asperelloids</i> and earthworm (<i>Eudrilus eugeniae</i>) 5 t/h]
T ₃	C ₃ [Predecomposed Sugarcane trash, <i>Trichoderma asperelloids</i> and Microbial consortium 5 t/h]
T ₄	C ₄ [Predecomposed Sugarcane bagasse, <i>Pleurotus florida</i> and earthworm (<i>Eudrilus eugeniae</i>) 5 t/h]
T ₅	C ₅ [Predecomposed Sugarcane bagasse, <i>Trichoderma asperelloids</i> and earthworm (<i>Eudrilus eugeniae</i>) 5 t/h]
T ₆	C ₆ [Predecomposed Sugarcane bagasse, <i>Trichoderma asperelloids</i> and Microbial consortium 5 t/h]

Statistical analysis

The data obtained from various observations on 30, 60 and 90 DAS were analyzed statistically using One-way and Two-way ANOVA.

RESULTS AND DISCUSSION

Biometric characters

The results of the present study predicted the effect of composts by different treatments on biometric characters of Brinjal (*Solanum melongena* L.). A highest shoot length was observed in T₅ treatment (32.9, 55.3 and 98.5 cm) which is followed by T₂ treatment (29.5, 51.4 and 93.7 cm) and control (17.3, 33.5 and 75.3 cm) on 30, 60 and 90 days after sowing (DAS). A significant increase in root length was observed in T₅ treatment (17.85, 25.10 and 33.30 cm) followed by T₂ treatment (16.30, 22.85 and 30.75 cm) and compared to the control (8.35, 13.40 and 21.30) 30, 60 and 90 days after sowing. Maximum number of leaves was observed in T₅ treatment (21.33, 50.00 and 77.00) when compared to the T₂ treatment (19.33, 46.00 and 72.67) and control (9.00, 26.00 and 54.67) 30, 60 and 90 days after sowing as shown in Table 1.

The fresh weight of the plant increase in T₅ treatment (31.10, 48.30 and 55.55 g) which is followed by T₂ treatment (28.90, 45.35 and 52.20 g) when compared to the control (16.70, 27.05 and 34.65 g) 30, 60 and 90 days after sowing. A significant increase in dry weight was observed in T₅ treatment (7.10, 7.95 and 9.30 g) followed by T₂ treatment (6.75, 7.45 and 8.80 g) and control (3.75, 5.30 and 5.50 g) on 30, 60 and 90 days after sowing as shown in Table 2. Similar increase in results were also observed in number of flowers in T₅ treatment (27.50 and 55.50) when compared to T₂ treatment (25.50 and 52.50) and control (12.50 and 38.00) 60 and 90 days after sowing. A highest number of branches was observed in T₅ (22.50 and 27.50) treatment which is followed by T₂ treatment (20.50 and 25.00) when

compared to the control (9.50 and 14.00) 60 and 90 days after sowing as shown in Table 3.

The results par with Manimegala and Gunasekaran (2020) who observed significantly higher plant height, number of leaves, number of branches and number of flowers recorded at 60 and 90 DAS of the crop with the application of treatment vermicompost and NPK fertilizer in eggplant (*Solanum melongena* L.). Similar work was reported by Tensing Baliah and Muthulakshmi (2017) who observed that the application of the vermicompost increase

the shoot length (33.5 cm), root length (22.6 cm), number of leaves (30), fresh weight (4.41 g) and dry weight (1.29 g) in okra (*Abelmoschus esculentus* L.) (El-Mohamedy *et al.*, 2015). A significant increase in plant height, number of leaves, number of branches, fresh weight and dry weight was observed in bio-compost when compared to the control in potato (*Solanum tuberosum* L.) plants. The results coincides with Silpa and Vijayalakshmi (2022) who confirmed that the application of biocomposted cocoa shell and jack fruit peel waste increased the shoot length (175.83 cm),

Table 1: Effect of composted sugarcane trash and bagasse on the shoot length, root length and number of leaves on *Solanum melongena* L.

Treatment	Shoot length (cm)			Root length (cm)			Number of leaves		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
C	17.3	33.5	75.3	8.35	13.40	21.30	9.00	26.00	54.67
T ₁	21.3	39.5	80.4	9.60	16.15	24.00	9.67	30.67	56.67
T ₂	29.5	51.4	93.7	16.30	22.85	30.75	19.33	46.00	72.67
T ₃	25.4	48.2	93.3	14.25	21.40	28.60	15.67	42.00	68.33
T ₄	23.3	46.1	89.6	12.45	18.80	26.60	13.67	37.33	64.00
T ₅	32.9	55.3	98.5	17.85	25.10	33.30	21.33	50.00	77.00
T ₆	22.4	41.4	85.4	11.25	17.50	24.05	11.67	34.00	61.33
SED		0.34231			3.2812			3.81864	
Cd (p<0.05)		0.69096			66.62340			7.70813	

DAS- Days after sowing.

Table 2: Effect of composted sugarcane trash and bagasse on the fresh weight and dry weight of *Solanum melongena* L.

Treatment	Fresh weight (g)			Dry weight (g)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
C	16.70	27.05	34.65	3.75	5.30	5.50
T ₁	20.45	34.05	37.95	4.40	5.75	6.40
T ₂	28.90	45.35	52.20	6.75	7.45	8.80
T ₃	27.30	41.95	48.35	6.25	7.10	8.20
T ₄	24.95	39.35	45.20	5.65	6.75	7.50
T ₅	31.10	48.30	55.55	7.10	7.95	9.30
T ₆	22.70	35.95	41.10	5.20	6.30	7.10
SED		3.28126			0.52068	
Cd (p<0.05)		6.62340			1.05103	

DAS- Days after sowing.

Table 3: Effect of composted sugarcane trash and bagasse on the number of flowers and number of branches of *Solanum melongena* L.

Treatment	Number of flowers		Number of branches	
	60 DAS	90 DAS	60 DAS	90 DAS
C	12.50	38.00	9.50	14.00
T ₁	15.50	42.00	12.00	16.50
T ₂	25.50	52.50	20.50	25.00
T ₃	23.00	50.50	18.00	22.50
T ₄	21.00	47.50	16.00	21.00
T ₅	27.50	55.50	22.50	27.50
T ₆	18.00	44.50	14.50	18.50
SED		3.72678		3.57238
Cd (p<0.05)		7.63417		7.31789

DAS- Days after sowing.

Table 4: Effect of composted sugarcane trash and bagasse on the yield parameters of *Solanum melongena* L.

Treatment	Number of fruits	Fruit length (cm)	Single fruit weight (g)	Fruit yield per plant (kg)	Fruit yield per plot (kg)
	90 DAS	90 DAS	90 DAS	90 DAS	90 DAS
C	14.50	11.30	57.45	7.30	25.10
T ₁	16.00	12.20	70.60	7.85	27.30
T ₂	20.50	15.40	91.80	10.00	35.35
T ₃	19.50	14.70	88.85	9.50	33.15
T ₄	18.00	13.95	80.65	9.30	30.85
T ₅	22.00	15.70	93.15	10.35	37.25
T ₆	17.00	13.05	75.50	8.20	29.10
SEd	6.5756	6.0387	7.0298	0.8153	0.6350
CD (p<0.05)	14.1047	12.9531	15.0792	1.7489	1.3620

DAS- Days after sowing.

root length (39.23 cm) and number of leaves (37.50) in *Vigna unguiculata* (L.) Walp. The present study was supported by Pinky and Vijayalakshmi (2022) who reported that the application of vermicompost increased the shoot length, root length, fresh weight and dry weight in black gram (*Vigna mungo* L.). The present study is in correlation with Sardoei (2014) reported that the application of vermicompost increase of plant height (26.87), fresh weight (85.43) and dry weight (13.78) in marigold (*Calendula officinalis*).

The present study was also in agreement where an increase in plant height (32.56 cm) was noted with the application of T₃- Azolla vermicompost supplemented with 50% NPK, was observed Palia *et al.* (2021).

Yield parameters

The maximum number fruits was observed in T₅ treatment (22.00) followed by T₂ treatment (20.50) when compared to the control (14.50). The fruit length and single fruit weight are significantly increased in T₅ treatment (15.70 cm and 93.15 g) when compared to the T₂ treatment (15.40 cm and 91.80 g) and control (11.30 cm and 57.45 g). The fruit yield per plant and fruit yield per plot was observed maximum in T₅ treatment (10.35 and 37.25 kg) which is followed by T₂ treatment (10.00 and 35.35 kg) and control (7.30 and 25.10 kg) on 90 days after sowing as shown in Table 4.

The present results also coincide with the previous findings Rahman *et al.* (2012) that bio-compost + cowdung compost + NPK fertilizers had significant positive impact on number of fruit (62.38), fruit length (23.98) and yield per plant (24.49) in chilli. The present study was correlated with the findings of Gandhi and Sivagama Sundari (2012) who reported that the application of vermicompost increase number of fruits (6-12) in brinjal plant (*Solanum melongena*). Mamta *et al.* (2012) A significant increase in total yield per plant with the application of T₄- pv 2.5 t/ha + FYM 6.25 t/ha in *Solanum melongena*.

The results was on par with Mullaimaran and Haripriya (2016) who confirmed that the application of organic manures increase number of fruits, single fruit weight, fruit yield per plant and fruit yield per plot in tomato. The present study coincides with the result of Kashem *et al.* (2015) in tomato (*Solanum lycopersicum* L.) which showed an increase number of fruits in vermicompost and NPK

fertilizers. Similar work was reported by Saraswathy and Prabhakaran (2014) observed that the application of vermicompost increase number of fruits (36.57), fruit weight (71.65 g), fruit yield per plot (28.82 kg) in tomato (*Lycopersicum esculentum* Mill.).

CONCLUSION

The study revealed that the integration of sugarcane trash and bagasse had shown an enhancing effect on growth and yield of brinjal. On the basis of results, it is concluded that the vermicompost prepared from the test substrates viz. C₅ [Predecomposed Sugarcane bagasse, *Trichoderma asperelloids* and earthworm (*Eudrilus eugeniae*) 5 t/h] revealed beneficial outcomes with improved effects on the quality attributes of Brinjal. Thus, the study indicates that the vermicompost can be utilized effectively for sustainable crop production.

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Conflict of interest

Authors have no conflict of interest regarding this article.

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