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## *Appendices*

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**APPENDICES****APPENDIX – I**  
**ESTIMATION OF LIGNIN**  
**(Goering and Vansoest, 1975)****Principle**

Refluxing the sample material with acid detergent solution removes the water soluble and materials other than the fibrous component. The left out material is weighed after filtration, dried, treated with 72% H<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>SO<sub>4</sub> (W/V)
- Acetone
- Round Bottom Flask and Refluxing Set
- Muffle Furnace
- Sintered Glass Crucible – G2

**Procedure****A. Acid Detergent Fibre (ADF)**

- 1gm 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 colourless.

**Method**

- 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 hrs.
- 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<sub>2</sub>SO<sub>4</sub> and followed the steps from 2 - 5.

### Calculation

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

## APPENDIX – II

### ESTIMATION OF CELLULOSE

(Updegroff, 1969)

### Principle

Cellulose undergoes acetolysis with acetic/nitric reagent forming acetylated cello dextrins which get dissolved and hydrolyzed to form glucose molecules upon treatment with 67% H<sub>2</sub>SO<sub>4</sub>. 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 0.1 g 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% H<sub>2</sub>SO<sub>4</sub> 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 - III**  
**ESTIMATION OF ORGANIC CARBON (%)**  
**WET CHROMIC ACID OXIDATION METHOD**  
**(Walkey and Black, 1934)**

**Principle**

Organic carbon present in organic matter is oxidised by chromic acid in the presence of conc. H<sub>2</sub>SO<sub>4</sub>. Potassium dichromate on reaction of H<sub>2</sub>SO<sub>4</sub> provides nascent oxygen which combines with carbon and form CO<sub>2</sub>. The H<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 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<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>SO<sub>4</sub> was added and the volume was made up to one litre.
- Conc. H<sub>2</sub>SO<sub>4</sub>
- 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 K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was added and mixed well by swirling the

flask. Added 20ml of conc. H<sub>2</sub>SO<sub>4</sub> 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 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 color proceeds the dull green shifted to turbid blue 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 <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	= 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 <sub>4</sub> reduces 10ml of 1N K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	
Therefore, Y ml of FeSO <sub>4</sub> reduces Y/X* 10ml	
Hence actual quantity of 1N K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> used for oxidation of organic matter	
1ml of 1N K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	= 10 - (10 x Y/X) = 0.003g of 'C'
Therefore 10 - (10 x Y/X) ml of 1N K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	= 10 - (10 x Y/X) x 0.003
This is present in 0.5g of soil	
Therefore in 100g	= 10 - (10 x Y/X) x 0.003 x 100/0.5
Organic matter (surface soil)	= organic carbon x 1.724
Organic matter (sub surface soil)	= organic carbon x 2.5

**APPENDIX - IV**

**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**

1. Diacid mixture: 4:1 (w/w) ratio of concentrated sulphuric acid and concentrated perchloric acid.

2. Mixed indicator: 0.5g bromocresol green and 1g of methyl red were dissolved in 100ml of 90% ethyl alcohol.
3. 40% sodium hydroxide solution.
4. 2% boric acid.
5. Concentrated sulphuric acid (0.02 N).

**Procedure**

1. 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.
2. Complete digestion was ensured by adding one drop of perchloric acid and the contents turns colourless like water.
3. The volume was made upto 100ml with distilled water.
4. 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.
5. To the distillation apparatus, 10ml of 40% sodium hydroxide was added and steam distilled. The distillate was collected until no more ammonia was evolved.
6. 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).                             |

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**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 volume.
- 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 hours 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 °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 <sub>3</sub> 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	= 0.0005g P <sub>2</sub> O <sub>5</sub>
(a-b) ml of 0.1619N KOH	= 0.0005 x (a-b) x gP <sub>2</sub> O <sub>5</sub>
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 g of sample	
Therefore, in 100 g	= 0.0005 x (a-b) x 500/200 x 100/W
Percentage of P <sub>2</sub> O <sub>5</sub> on moisture free basis	
= 0.0005 x (a-b) x 500/200 x100/W x 100/(100 – M)	
(M – Moisture content of the sample)	

**APPENDIX - VI**  
**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 characteristics 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 at 100 on the scale 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	= $A/10^6$ g of K
Therefore, in 250 ml of the solution	= $A/10^6 \times 250$
This was present in 50 ml of HCl extract	
Therefore, in 500 ml	= $A/10^6 \times 250 \times 500/50$ g
This was present in W g of sample	
Therefore, in 100 g	= $A/10^6 \times 250 \times 500/50 \times 100/W$ g
Percentage of K on moisture free basis	
	= $A/10^6 \times 250 \times 500/50 \times 100/W \times 100/(100 - M)$
	(M – Moisture content of sample)

## APPENDIX- VII

### 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 filtrate 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 R<sub>2</sub>O<sub>3</sub> estimation = 50 ml  
 Volume of R<sub>2</sub>O<sub>3</sub> filtrate made upto = 250 ml  
 Volume of R<sub>2</sub>O<sub>3</sub> 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 formagnesium 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

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

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

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**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 - 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  $\mu\text{g}$  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 10minutes. Then, 0.5ml 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/g or 100 g sample.

$$= \frac{\text{mg of protein}}{\text{volume of test standard}} \times \text{concentration of the standard}$$

**APPENDIX – IX****ESTIMATION OF CARBOHYDRATE (Hedge and Hofreiter, 1962)****ANTHRONE METHOD****Principle**

Carbohydrates are first hydrolysed 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: 200 mg anthrone was dissolved in 100 ml of ice cold 95% H<sub>2</sub>SO<sub>4</sub> and it was prepared fresh before use.
- Standard glucose: (Stock) 100 mg 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**

100 mg of the sample (leaf) was taken in a boiling tube with 5ml of 2.5 N HCl, hydrolysed 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**

$$\text{Amount of carbohydrate present in 100 mg of the sample} = \frac{\text{mg of glucose}}{\text{volume of test sample}} \times 100$$

**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 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 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 was 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<sup>-1</sup> tissues by using the following equations.

$$\begin{aligned} \text{(i) Chlorophyll 'a' mg g}^{-1} \text{ tissues} &= 12.7A(663) - 2.69 A(645) \times \frac{V}{1000 \times W} \\ \text{(ii) Chlorophyll 'b' mg g}^{-1} \text{ tissues} &= 22.9A(645) - 4.68 A(663) \times \frac{V}{1000 \times W} \\ \text{(iii) Total Chlorophyll mg g}^{-1} \text{ tissues} &= 20.2 A(645) - 8.02 A(663) \times \frac{V}{1000 \times W} \end{aligned}$$

Where,

- A = absorbance of specific wavelengths  
 1. = final volume of chlorophyll extract in 80 % acetone.  
 2. = 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 stopped tubes in dessicator.
- 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

Lb concentration (mM) =  $A_{556} - A_{539} \times 2D/23.4$  Where, D is the initial dilution.

(The calculation is based upon the equation  $E = 23.4 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$ )

## APPENDIX – XII

### PRELIMINARY PHYTOCHEMICAL SCREENING

(Shaikh and Patil, 2020)

#### 1. Detection of alkaloids

- Dragendorff's/Kraut's test: Few ml of filtrate + 1-2 mL *Dragendorff's reagents* appearance of reddish-brown precipitate indicative of alkaloids
- Mayer's/Bertrand's/ Valser's test: Few ml of filtrate + 1-2 drops of *Mayer's reagent* (Along the sides of test tube) appearance of creamy white/yellow precipitate indicative for alkaloids

#### 2. Detection of flavonoids

- Alkaline reagent test: 1mL extract + 2mL of 2% NaOH solution (+ few drops dil.HCl) appearance of intense yellow color, becomes colorless on addition of diluted acid is suggestive of flavonoids

- b. Plant extract + 10% ammonium hydroxide sol appearance of yellow fluorescence.
- c. Lead acetate test: 1 ml of plant extract with few drops of 10% lead acetate solution appearance of yellow precipitate indicative of flavonoids.
- d. Shinoda's test/Mg - hydrochloride reduction test: Plant extract is dissolved in 5ml alcohol + Fragments of magnesium ribbon + few drops of conc. HCl appearance of pink to crimson colored solution indicative of flavonoids.

### 3. Detection of Sterols

- a. Libermann - Burchard's test: 50 g extra is dissolved in 2ml acetic anhydride + 1-2 drops of con. H<sub>2</sub>SO<sub>4</sub> (along the side of test tube). The appearance of array of color change is indicative of sterols.

### 4. Detection of Terpenoids

Libermann – Burchard's test: 2ml of chloroform + 5 ml plant extract, (evaporated on water bath) + 3ml conc. H<sub>2</sub>SO<sub>4</sub> (boiled on water bath) appearance of grey color solution suggestive of Terpenoids.

### 5. Detection of Anthroquinones

Bomtrager's test: 10ml of 10% ammonia solution + few ml of filtrate (shaken vigorously for 30 seconds) appearance of pink, violet, or red colored solution indicative of Anthroquinones.

### 6. Detection of Anthocyanins

HCl test: 2ml plant extract + 2ml of 2N HCl (+ few ml of ammonia) appearance of pink red sol. which turns blue violet after addition of ammonia indicative of anthocyanins.

### 7. Detection of Proteins

- a. Biuret test: 2ml of filtrate + 1 drop of 2% copersulphate sol. + 1ml of 95% of ethanol + KOH pellets appearance of pink colored solution indicates presence of proteins. (in ehanolic layer)
- b. Ninhydrin test: 2ml of filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200 ml acetone) appearance of purple colored solution indicates the presence of proteins, (amino acids).
- c. Xanthoproteic test: Plant extract + few drops of conc. Nitric acid appearance of yellow colored solution suggestive of proteins.

### 8. Detection of Phenolic compounds

- a. Ferric chloride test: Extract aqueous solution + few drops 5% ferric chloride solution result in forming dark green/ bluish black color and indicates the presence of Phenolic compounds.
- b. Lead acetate test: Plant extract is dissolved in 5ml distilled water + 3ml of 10% lead acetate solution appearance of white precipitate the presence of Phenolic compounds.

- c. Ellagic Acid Test: plant extract aqueous solution + 5% glacial acetic acid + 5% sodium nitrite solution appearance of solution turns muddy/ Niger brown precipitate the presence of Phenolic compounds.

### 9. Detection of Quinones

- a. Alcoholic KOH test: 1ml of plant extract + few ml of alcoholic potassium hydroxide z: 1ml of filtrate + 1.5 ml of glacial acetic acid + 1 drop of 5% ferric chloride + con. H<sub>2</sub>SO<sub>4</sub> (along the side of test tube) formation of a blue colored solution suggestive of cardiac glycosides (in acetic acid layer)
- b. Bromine water test: Plant extract + few ml of bromine water appearance of yellow precipitate suggestive of cardiac glycosides.
- c. Baljet's test: 2 ml of extract + a drop of Baljet's reagent appearance of yellow orangecolor indicates the presence of cardiac glycosides.

### 15. Detection of Glycosides

- a. Modified Bontrager's test: Plant extract + ferric chloride solution + boil for 5min + cooled + equal volume of benzene layer is separated + Ammonia solution formation of rose - pink to blood red colored solution indicate the presence of glycosides.
- b. Aqueous NaOH test: Alcoholic extract + dissolved in 1 ml of water + few drops of aqueous NaOH solution forms a yellow color.

**16. Detection of Lignin:** Labat test: Extract solution + Gallic acid forms olive green color.

**17. Detection of Coumarins:** NaOH test: Plant extract + 10 % NaOH + Chloroform form a yellow color.

### 18. Detection of volatile oils

Fluorescence test: 10ml of extract, filtered till saturation, exposed to UV light. Appearance of bright pinkish fluorescence indicates the presence of volatile oils.

### 19. Detection of carotenoids

Carr-Price reaction: 10ml of extract evaporated to dryness + 2-3 drops of saturated solution of antimony tri chloride in chloroform appearance of blue - green color eventually changing to red indicate the presence of carotenoids.

## APPENDIX – XIII

### ANTIOXIDANT ACTIVITY

#### 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 diphenylpicryl hydrazine. This can be identified by the conversion of purple to light yellow colour.

**Reagents**

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

**Procedure**

The extracts (20 µl) were added to 0.5ml of methanolic solution of DPPH and 0.48 ml 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 518 nm in a spectrophotometer. The radical scavenging activity was calculated as follows

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

**APPENDIX – XIV****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 decrease at this wavelength. This property is utilized to quantify their H<sub>2</sub>O<sub>2</sub> scavenging ability.

**Reagents**

- Phosphate buffer (0.1M, pH 7.4)
- H<sub>2</sub>SO<sub>4</sub> (40mM) in phosphate buffer

**Procedure**

A solution of H<sub>2</sub>O<sub>2</sub> (40 mM) was prepared in phosphate buffer. Plant extracts at the concentration of 5µl were added to H<sub>2</sub>O<sub>2</sub> solution (0.6 ml) and the final volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230 nm in a spectrophotometer. A blank solution containing phosphate buffer, without H<sub>2</sub>O<sub>2</sub> was prepared. The extent of H<sub>2</sub>O<sub>2</sub> scavenging of the plant extracts was calculated as

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

A<sub>0</sub> - Absorbance of control; A<sub>1</sub> - Absorbance in the presence of plant extracts

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**APPENDIX – XV**  
**NITRIC OXIDE RADICAL SCAVENGING ACTIVITY**  
**(Green *et al.*, 1982)**

**Principle**

At physiological pH, sodium nitroprusside generates nitric oxide which interacts with O<sub>2</sub> 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 colourchromophore was developed and the absorbance was read at 546 nm.

**APPENDIX – XVI**  
**REDUCING POWER ASSAY**  
**(Oyaizu, 1986)**

**Principle**

Substances, which have reduction potential, react with potassium ferricyanide (Fe<sub>3</sub><sup>+</sup>) to form potassium ferrocyanide (Fe<sub>2</sub><sup>+</sup>), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Potassium ferricyanide + Ferric chloride → 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.

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**APPENDIX – XVII**  
**ASSESSMENT OF ANTIBACTERIAL ACTIVITY**  
**(WELL DIFFUSION METHOD)**  
**(Bauer *et al.*, 1996)**

**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  $2 \times 10^8$  CFU/ml for *Escherichia coli*, *Vibrio cholera* 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 jar 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.
4. The media was punctured by making a well of 6 mm in diameter and filled with 30 µl of a sample. Further the petriplates were placed inversely for complete diffusion and inhibition zones 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.

**APPENDIX – XVIII**  
**ESTIMATION OF AVAILABLE NITROGEN IN SOIL**  
**ALKALINE PERMANGANATE METHOD**  
**(Subbiah and Asijia, 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  $\text{KMnO}_4$  in the presence of  $\text{NaOH}$  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  $\text{H}_2\text{SO}_4$ .

**Reagents**

- 0.32%  $\text{KMnO}_4$  solution (3.2 g of  $\text{KMnO}_4$  dissolved in one litre of distilled water).
- 2.5%  $\text{NaOH}$  solution (25 g of  $\text{NaOH}$  dissolved in one litre of distilled water).
- 2% boric acid (20 g of boric acid dissolved in one litre of distilled water).
- N/50  $\text{H}_2\text{SO}_4$  (30 ml of Conc.  $\text{H}_2\text{SO}_4$  is diluted to one litre with distilled water and standardized by titration with N/10  $\text{Na}_2\text{CO}_3$ . This gives N/10  $\text{H}_2\text{SO}_4$ . From this N/50  $\text{H}_2\text{SO}_4$  is prepared by dilution.
- Double indicator bromocresol green (0.5 g) 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%  $\text{KMnO}_4$  and 100 ml 2.5%  $\text{NaOH}$  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  $\text{H}_2\text{SO}_4$ .

**Calculation**

Weight of the soil taken	= 20g
Volume of N/50 $\text{H}_2\text{SO}_4$	= X ml (titer value)
1 ml of N/10 $\text{H}_2\text{SO}_4$	= 0.0014 gN
Therefore 1 ml of N/50 $\text{H}_2\text{SO}_4$	= 0.00028gN
X ml of N/50 $\text{H}_2\text{SO}_4$	= 0.00028 * X gN
This is present in 20 gm of soil	
Therefore N present in Kg/Ha	= 0.00028 (X/20) * $10^6$

**APPENDIX - XIX**  
**ESTIMATION OF AVAILABLE PHOSPHORUS IN SOIL**  
**CALORIMETRY METHOD**  
**(BRAY 1 METHOD – Jackson, 1973)**

**Principle**

The combination of HCl and NH<sub>4</sub>F extracts acid soluble forms of phosphorus such as mono calcium phosphate. The fluoride ion has the special property of complexing Al<sup>+++</sup> and Fe<sup>+++</sup> 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 colorimetrically as available phosphorus.

**Reagents**

- NH<sub>4</sub>F solution (1N): 37g of NH<sub>4</sub>F was dissolved in 1 litre of distilled water.
- HCl (0.05N): 20.2 ml conc. HCl diluted 500 ml with distilled water.
- Bray No. 1 extractant [0.03 NH<sub>4</sub>F and 0.02 N HCl]: 15 ml of 1N NH<sub>4</sub>F and 25 ml of 0.5N HCl are mixed and the volume was upto 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 reciprocatory mechanical shaker for one minutes. 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 NaHCO <sub>3</sub>	= 50 ml
Volume of extractant solution used for Phosphorus estimation (aliquot)	= 5 ml
calorimeter reading	= T
Concentration of phosphorus read from standard graph for the reading T	= X ppm = X mg/ml

	= X/10 <sup>6</sup> g/ml
Therefore in 25 ml of solution	= X/10 <sup>6</sup> x 25g
This is present in 50 ml of the extractant solution and 5 g of soil	
Therefore available P <sub>2</sub> O <sub>5</sub> in kg/ha	= X x 25 x 50 x 2 x 10 <sup>6</sup> 10 <sup>6</sup> x 5 x 5

**APPENDIX – XX**  
**ESTIMATION OF AVAILABLE POTASSIUM IN SOIL**  
**FLAME PHOTOMETRY METHOD**  
**(Stanford and English, 1949)**

**Principle**

- The potassium ions in the exchange site are replaced with NH<sub>4</sub><sup>+</sup> and K<sup>+</sup> 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**

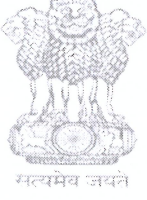
Transferred 5g of soil into a polythene shaking bottle. Added 25 ml of 1 N ammonium acetate and contents shaken in a mechanical reciprocating shaker for 5 minutes. Contents filtered through whatman No. 40 filter paper. Filterates were fed into the flame photometer and the readings recorded. Using standard curve available potassium content was calculated.

**Calculation**

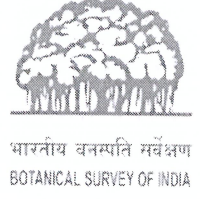
Weight of the soil taken	= 5 g
Volume of the extractant used	= 25 ml
Flame photometer reading	= T
Concentration of K in the standard curve	= X ppm = X mg/ml = X/10 <sup>6</sup> g/ml
Therefore, in 25 ml solution	= X/10 <sup>6</sup> x 25g
This is present in 5gm of soil	
Therefore, available K in soil in kg/ha	= X/10 <sup>6</sup> x 25 x 2 x 10 <sup>6</sup> /5

*Annexures*

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भारत सरकार  
GOVERNMENT OF INDIA  
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय  
MINISTRY OF ENVIRONMENT, FOREST AND CLIMATE CHANGE  
भारतीय वनस्पति सर्वेक्षण / BOTANICAL SURVEY OF INDIA  
दक्कन क्षेत्रीय केंद्र / DECCAN REGIONAL CENTRE  
हैदराबाद / HYDERABAD - 500 001  
तेलंगाना / TELANGANA



संख्या/No.: BSI/DRC/2022-23/ Identification/ 653

दिनांक/Date: 03/01/2023

सेवा मे/To

Miss. S. Hema  
Ph.D. Scholar  
Department of Botany,  
St. Joseph's College for Women,  
Visakapatnam, Andhra Pradesh-530004.

विषय: पौधों की पहचान के संबंध में।

Subject: Identification of plant materials regarding.

प्रिय कुमारी एस. हेमा, /Dear Ms. S. Hema

आपके पत्र दिनांक 29.12.2022 के संदर्भ में आपके द्वारा भेजी गई पौधों के नमूनों की पहचान **ट्रिगोनेला फोनम-ग्रैकम** एल. (फैबेसी) के रूप में की गई है। पहचान के बाद, पौधे का नमूना इसके साथ वापस कर दिया है।

With reference to your letter dated 29.12.2022, the plant material sent by you has been identified by the concerned expert as **Trigoneilia foenum-graecum** L. belongs to the family Fabaceae. After identification, the plant material is returned herewith.

धन्यवाद /Thanking you,

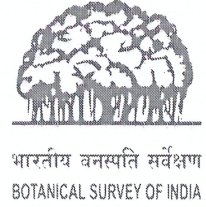
भवदीया/ Yours faithfully

एल. रासिंगम  
03/01/23

(एल. रासिंगम/ L. Rasingam)  
वैज्ञानिक-ई एवम् का. अ. /Scientist - 'E' & HoO



भारत सरकार  
GOVERNMENT OF INDIA  
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय  
MINISTRY OF ENVIRONMENT, FOREST AND CLIMATE CHANGE  
भारतीय वनस्पति सर्वेक्षण / BOTANICAL SURVEY OF INDIA  
दक्कन क्षेत्रीय केंद्र / DECCAN REGIONAL CENTRE  
हैदराबाद / HYDERABAD - 500 001  
तेलंगाना / TELANGANA



भारतीय वनस्पति सर्वेक्षण  
BOTANICAL SURVEY OF INDIA

संख्या/No.: BSI/DRC/2022-23/ Identification/ 580

दिनांक/Date: 23/11/2022

सेवा मे/To

Miss. S. Hema  
Ph.D. Scholar  
Department of Botany,  
St. Joseph's College for Women,  
Visakapatnam, Andhra Pradesh-530004.

विषय: पौधों की पहचान के संबंध में।

Subject: Identification of plant materials regarding.

प्रिय कुमारी एस. हेमा, /Dear Ms. S. Hema

आपके पत्र दिनांक 14.11.2022 के संदर्भ में आपके द्वारा भेजी गई पौधों के नमूनों की पहचान संबंधित विशेषज्ञ द्वारा इस प्रकार की गई है:

With reference to your letter dated 14 November 2022, the plant materials sent by you have been identified by the concerned expert as follows:

Filed No.1. **Vigna aconitifolia** (Jacq.) Maréchal belongs to the family Fabaceae,

Filed No.2. **Vigna mungo** (L.) Hepper belongs to the family Fabaceae,

Filed No.3. **Andrographis paniculata** (Burm.f.) Nees belongs to the family Acanthaceae.

पहचान के बाद, पौधे का नमूना इसके साथ वापस कर दिया है।/ After identification, the plant material is returned herewith.

धन्यवाद /Thanking you,

Yours faithfully

एल. रासिंगम  
23/11/22

एल.रासिंगम/ L. Rasingam

वैज्ञानिक-'ई' एवम् का.अ./Scientist - 'E' & HoO

*Publications*

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## Avinashilingam Institute for Home Science 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	The Effect of Paddy and Coffee Husk as the Organic Manure on the Vegetative and Yield Parameters of Moth Bean [ <i>Vigna aconitifolia</i> (Jacq) Marechal].	Agricultural Science Digest	Vol: 44(2), 260-264 2024.	Scopus Indexed
2	Impact of Paddy and Coffee Husk as the Bio Compost and its Effect on the Growth and Yield of Black Gram [ <i>Vigna mungo</i> (L.) Hepper].	Agricultural Science Digest	Vol: 44(1), 184-188 2024.	Scopus Indexed

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

Scholar : S. Keen

Supervisor & HOD : A. Vijayabala  
7/5/24

Checked By:

  
7/5/2024  
HoD /Dean of Respective School

⇨

The scholar Sr. Hema, S (Reg. No. 17 PHBOP001) has published her article in the following journal:

1. Agricultural Science Digest - indexed and active in Scopus from 2019 to present.

This may be considered.

J. J. → G. N.  
07.05.2024.



# The Effect of Paddy and Coffee Husk as the Organic Manure on the Vegetative and Yield Parameters of Moth Bean [*Vigna aconitifolia* (Jacq) Marechal]

S. Hema<sup>1</sup>, A. Vijayalakshmi<sup>1</sup>, Pinky Raihing<sup>1</sup>

10.18805/ag.D-5578

## ABSTRACT

**Background:** Amidst of emerging world with the new technologies we are in the dangerous condition regarding the disposal of agro industrial wastes which creates a serious problem. Organic manures can prevent and can enhance the productivity in the plants as well as can reduce the environmental issues.

**Methods:** The study was conducted in the month of September to November 2019 at St. Joseph's College Campus, Visakhapatnam, A.P. with the bio composting process of paddy and coffee husk along with *Pleurotus eous*, *Pleurotus florida*, *Trichoderma asperelloides* and *Eisenia fetida*, with 6 treatments. The plants were analyzed for root length, shoot length, number of leaves, number of nodules, fresh weight and dry weight of the plant on 20, 40 and 60 DAS and the yield parameters were analyzed on 90<sup>th</sup> day of the plant.

**Result:** The results showed a great increase in the treatment 6 (Pre decomposed coffee husk, consortium of *Pleurotus eous*, *P. florida*, *Trichoderma asperelloides* and *Eisenia fetida*) in root length (8.5, 12.9 and 16.1 cm) shoot length (15.2, 30.3 and 43.2 cm) number of leaves (11, 18 and 49) number of nodules (12, 20 and 9) fresh weight (4.83, 8.98 and 15.47 g) and dry weight (1.11, 3.56 and 4.37 g) when compared with the other treatments and control. The yield parameters also showed significant results in number of pods (27), length of the pod (5.7 cm), number of seeds/pod (6), weight of the seeds/pod (2.89 g), fresh weight of the pod (1.653 g) and dry weight of the pod (0.986 g).

**Key words:** *Eisenia fetida*, Mothbean, Organic manure, *Pleurotus eous*, *Pleurotus florida*, *Trichoderma asperelloides*.

## INTRODUCTION

Over the past century the attention is given to the agricultural science specially as a response to the excess growth of the global population, particularly the issues like food, crop and security and the technological advances resulting the many emerging issues and challenges to the future generations and among them the important challenge is the disposal of agro industrial wastes which is also a great hazard with its unorganized deposition which can cause environmental issues like pollution, landfills, eutrophication, economic losses, health risks and also climate changes by releasing harmful greenhouse gases (Khuriyati and Kumalasari, 2015).

Every year nearly 20 million tons of paddy is produced, which results in generating nearly 24 million tons of paddy straw and husk. If these are burnt then 4.4 million tons of paddyash is produced, which could be used in brick industry, steel and cement (Giddel and Jivani, 2007).

In India there are different commercial crops one of the important among them is coffee and it is the export crop which alone fetches a considerable and desirable foreign exchange to various countries. In India the southern states of Karnataka (71%), Kerala (21%) and Tamil Nadu (5%) overall produce 8,200 tons (Velmourougane *et al.*, 2010). According to the statistical data coffee productivity in India from financial year 2013 to 2020 by state shows Karnataka has taken the 1<sup>st</sup> place in producing the coffee and it is followed by Kerala, Tamil Nadu, Andhra Pradesh

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Email: hemasr9@gmail.com

**How to cite this article:** Hema, S., Vijayalakshmi, A. and Raihing, P. (2024). The Effect of Paddy and Coffee Husk as the Organic Manure on the Vegetative and Yield Parameters of Moth Bean [*Vigna aconitifolia* (Jacq) Marechal]. *Agricultural Science Digest*. 44(2): 260-264. doi: 10.18805/ag.D-5578.

**Submitted:** 24-02-2022 **Accepted:** 20-06-2022 **Online:** 15-07-2022

and Odisha. The coffee plantations, coffee pulp and seed husk are formal to be more hazardous and major solid water which are gained during its processing about 1 ton of husk is generated during its dry processing since this is lingo cellulosic waste and it as hazardous (Aranda and Barois, 2000).

Use of paddy husk and coffee husk which is fibrous in nature and also they are fatal to cattle feeding and filling up of the land with these wastes is more hazardous to the environment and is wrong way of disposing of the waste. Moth bean is one of the most drought resistant crop grows in semi arid and arid regions of Gujarat, Karnataka, Rajasthan, Maharashtra and Haryana and its yield is much

less as compared to other legume crops, hence it is necessary to enhance the production specially by applying organic manure, biofertilizer and micronutrients in various combinations as the need is increasing to reduce the usage of chemical fertilizer and increase the use of organic fertilizer to improve the physico-chemical as well as the soil fertility the supply of the nutrients in the plant (Sipai *et al.*, 2018).

**MATERIALS AND METHODS**

**Pot culture study of moth bean**

The experimental pot was filled with 7 kgs of red sandy loam soil and control also was maintained. The seeds of *Vigna aconitifolia* (Jacq) Marechal were obtained from Jaipur Rajasthan Agriculture institute. Viable seeds were used for pot culture studies with 3 replications of each treatment and it was conducted during September-November 2019 at St. Joseph's college campus, Visakhapatnam, A.P. at the latitude 17.719185 and longitude 83.286938.

The treatments are as follows:

C - Control.

T<sub>1</sub>- Pre decomposed paddy husk, *Pleurotus eous* and *Eisenia fetida* (5 t/ha).

T<sub>2</sub>- Pre decomposed paddy husk, *Pleurotus florida* and *Eisenia fetida* (5 t/ha).

T<sub>3</sub>- Pre decomposed paddy husk, consortium of *Pleurotus eous*, *Pleurotus florida*, *Trichoderma asperelloides* and *Eisenia fetida* (5 t/ha).

T<sub>4</sub>- Pre decomposed coffee husk, *Pleurotus eous* and *Eisenia fetida* (5t/ha).

T<sub>5</sub>- Pre decomposed coffee husk, *Pleurotus florida* and *Eisenia fetida* (5 t/ha).

T<sub>6</sub>- Pre decomposed coffee husk, Consortium of *Pleurotus eous*, *P. florida*, *Trichoderma asperelloides* and *Eisenia fetida* (5 t/ha).

Vegetative parameters such as shoot length, root length, number of leaves, number of nodules, fresh weight and dry weight on 20, 40 and 60 days after sowing (DAS) and on 90<sup>th</sup> day yield parameters of the plant such as number of pods/plant, length of the pod, number of seeds/pod, weight of the seeds/pod, pod fresh weight and pod dry weight were evaluated and the inference was subjected to statistical analysis of one way and two way ANOVA with the software Sigma stat 3.1.

**RESULTS AND DISCUSSION**

**The effect of paddy and coffee husk as the organic manure on the vegetative parameters of moth bean [*Vigna aconitifolia* (Jacq) marechal]**

The present study shows the influence of the agro industrial wastes paddy and coffee husks and its effect on the

**Table 1:** Effect of paddy and coffee husk as organic manure on the vegetative parameters of moth bean on 20, 40 and 60 DAS.

Treatments	Root length (cm)			Shoot length (cm)		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
C	3.9	9.7	12.4	9.2	19.5	27.2
T1	5.5	10.5	13.2	13.1	22.1	33.4
T2	6.2	11.3	14.1	11.9	24.4	32.9
T3	7.1	12.2	15.8	14.9	29.2	41
T4	4.7	11.4	14.6	14.3	20.9	34.1
T5	5.8	11.9	15	13.7	23.9	40.2
T6	8.5	12.9	16.1	15.2	30.3	43.2
SEd		0.34365			0.39561	
CD (p<0.05)		0.69368**			0.79856**	

\*\*Significant at (p<0.05); DAS - Days after sowing.

**Table 2:** Vegetative parameters of moth bean with the influence of paddy and coffee husk as the organic manure.

Treatments	Number of leaves (cm)			Number of nodules		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
C	7	11	21	5	10	3
T1	8	13	26	7	13	5
T2	8	12	24	8	16	6
T3	9	16	45	10	19	8
T4	7	13	25	8	15	5
T5	8	12	27	9	14	7
T6	11	18	49	12	20	9
SEd		0.40053			0.44186	
CD (p<0.05)		0.80849**			0.89191**	

\*\*Significant at (p<0.05); DAS - Days after sowing.

vegetative growth and yield parameters of moth bean (*Vigna aconitifolia*(Jacq) Marechal) the results are as shown in Table (1-4).

Root length was observed during 20, 40 and 60 DAS and it is found to be high in treatment 6 (Pre decomposed Coffee husk, consortium of *Pleurotus eous*, *P.florida*, *Trichoderma asperelloides* and *Eisenia fetida* (8.5,12.9 and 16.1 cm) among all the treatments and it is followed by the treatment 3 (Pre decomposed paddy husk, consortium of *Pleurotus eous*, *P. florida*, *Trichoderma asperelloides* and *Eisenia fetida*.) (7.1, 12.2 and 15.8 cm) when compared to control (3.9, 9.7 and 12.4 cm).

Significantly the increased growth of the Shoot length also observed in Treatment 6 (15.2, 30.3 and 43.2 cm) followed by treatment 3 (14.9, 29.2 and 41.0 cm) compared to control (9.2, 19.5 and 27.2 cm). A marvelous improvement in number of leaves was observed in treatment 6 (11, 18 and 49) (Table 2) when compared to treatment 3 (9, 16 and 45) and control (7, 11 and 21).

Number of nodules as it is showed in Table 2 there was increase in 20 DAS to 40 DAS later there is decreasing trend found at 60 DAS and the highest number of nodules obtained in treatment 6 (12, 20 and 9) followed by treatment 3 (10, 19 and 8) compared to control (5, 10 and 3).

### Fresh weight and dry weight

According to Table 3 the maximum fresh weight (4.83,8.98 and 15.47 g) and dry weight (1.11, 3.56 and 4.37 g) of test crop was found in treatment 6 and it is followed by treatment 3 (4.11,7.99 and 14.97 g) (0.99, 3.32 and 4.19 g) compared to the control (2.97,6.02 and 10.79 g), (0.45,2.14 and 3.09 g). The present study is in par with the results of Raihing and Vijayalakshmi, (2021) where the effect of fruit and vegetable vermicompost on lablab (*Lablab purpureus* (L) Sweet) showed the highest shoot length in T8 Treatment (113.7, 124.5 and 135 cm) on 15, 35 and 55 days after sowing (DAS).

Manivannan *et al.* (2009) found in *Phaseolus vulgaris*, when applied with vermicompost and NPK gave good results in growth parameters such as root length (22.5 cm) shoot length (69.1 cm) and number of nodules (39).

According to Haireddy and Joy Dawson, (2021) during harvest time the vermicompost application (6 t/ha) with *Rhizobium* and *Azospirillum* has resulted in the maximum growth of plant height (40.20 cm) comparatively it is superior to the other treatments. The influencing factors are always for the growth of the plant height and it is of the nutrients which are from the organic manures and biofertilizers work as the available nutrients for the best growth of the plant height (Darshanth and Singh, 2014).

**Table 3:** The effect of paddy and coffee husk as the organic manure on the vegetative parameters of moth bean on 20, 40 and 60 DAS.

Treatments	Fresh weight (g)			Dry weight (g)		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
C	2.97	6.02	10.79	0.45	2.14	3.09
T1	3.54	6.85	11.34	0.54	2.32	3.74
T2	3.74	7.61	12.53	0.69	2.65	4.1
T3	4.11	7.99	14.97	0.99	3.32	4.19
T4	3.71	6.76	12.25	0.53	2.5	4
T5	3.93	7.01	13.41	0.73	2.42	4.03
T6	4.83	8.98	15.47	1.11	3.56	4.37
SEd		0.39463			0.36884	
CD (p<0.05)		0.79659**			0.74453****	

Significant at (p<0.05); DAS- Days after sowing.

**Table 4:** Influence of paddy and coffee husk as the organic manure on the yield parameters of the moth bean at 90 DAS.

Treatments	Number of pods /plant	Length of the pod (cm)	Number of seeds /pod	weight of the seeds/pod (g)	Pod fresh weight (g)	Pod dry weight (g)
C	15	3.4	4.0	2.01	0.986	0.521
T1	17	4.3	5.0	2.16	1.312	0.663
T2	18	4.1	4.0	2.42	1.283	0.843
T3	23	5.0	5.0	2.68	1.541	0.946
T4	16	3.8	5.0	2.33	1.041	0.713
T5	19	4.2	4.0	2.12	1.033	0.638
T6	27	5.7	6.0	2.89	1.653	0.986
SEd	0.1764	0.6355	0.2588	0.1633	0.1383	0.0319
CD (p<0.05)	0.3843	1.3631	0.5552	0.3503	0.2966	0.0685

\*\* Significant at (p<0.05); DAS- Days after sowing.

Highest number of leaves (13.66) were found in leafy vegetables with the treatment of dead sheep compost in comparison with control (8.66) (Al-Sabbagh *et al.*, 2020). Similar results were observed by Kavya *et al.* (2021) that on applying RDF + Fe 0.5% + Zn 0.5% + Mn 0.5% recorded maximum height of the plant (42.42) at 60 DAS in *Vigna radiata* L.

#### **Influence of paddy and coffee husk as the organic manure on the yield parameters of the moth bean [*Vigna aconitifolia* (Jacq) Marechal] at 90 DAS**

The yield concept always based on the quantitative idea of the fertilizer is based on yield and nutritional requirement of the crop and the nutrient of the soil available with that of the applied fertilizer (Regar and Singh 2014; Sakarvadia *et al.*, 2021).

The remarkable number of pods/plant, length of pod, number of seeds/pod, weight of the seeds/pod, pod fresh weight and pod dry weight found in the treatment 6 when it is compared to the control and all the other treatments, the Treatment 6 is followed by 3<sup>rd</sup> Treatment in its increase. The maximum number of pods/plant (27) number of seeds/pod (6) length of the pod (5.7 cm), weight of the seeds/pod (2.89 g), pod fresh weight (1.653 g) and pod dry weight (0.986 g) was found high among all the treatments and the minimum results were found in control as it is shown in Table 4 similar findings were found with the work of Ruheentaj and Sarawad, (2020) with the application of vermicompost on the growth of *Vigna aconitifolia* (Jacq) Marechal recorded highest yield (625.0 kg/ha) and significant results also found with the application of vermicompost and FYM in the increase of pod numbers (61.47/plant), pod length (8.67 cm) seed weight (1.96 g) it also coincides with that of findings of Sadashivanagowda *et al.* (2017) with incorporation of vermicompost in black gram yield is noticed to be highest. According to Sipai *et al.* (2018) with the incorporation of prophenophenol 50% EC at 0.05% and *Helicoverpa* in the module 4 showed great results in the growth of the moth bean yield seeds (1316 kg/ha) when compared with all the other modules.

Similar results were observed by Silpa and Vijayalakshmi, (2021) with the inclusion of Jackfruit peel vermicompost resulted in the highest yield of *Vigna unguiculata* (L) Walp in the treatment 8 such as number of pods (21), length of the pod (16.50 cm), weight of the seeds (1.68 g), pod fresh weight (5.711 g) and dry weight (2.398 g). The results of this particular study also is similar to that of Hasan *et al.* (2021) with the application of N and P fertilizer on Groundnut plants and found that the plant height (20.65) increased along with the number of pods 74.28% in treatment 15. Changkija and Gohain, (2018) obtained the significant results in increasing the grain weight (12.07 g) of soy bean with the application of poultry manure in treatment 6. Similar to these results Zebire and Gelgelo, (2019) reported that there was increase in the yield (74.2 g) of haricot bean with application of P fertilizer.

## **CONCLUSION**

The present study clearly reveals the significant effect of paddy and coffee husk compost as organic manure on Moth bean and the significant values proved that it is effective bio compost specially the Treatment 6 which showed the maximum increase in the vegetative growth as well as yield parameters of the test plant. In conclusion we suggest that the biocomposting of agro industrial waste is the effective tool to protect the environment by lessening the harmful effects of the waste, as well as to increase the crop production and it can be suggested to the farmers to use the bio compost as the organic manure for better income as well as better results in the crop.

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## **Conflict of interest**

I declare that authors have no conflict of interest regarding this article.

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# Impact of Paddy and Coffee Husk as the Bio Compost and its Effect on the Growth and Yield of Black Gram [*Vigna mungo* (L.) Hepper]

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## ABSTRACT

The recent developments of urban areas change the climate in the ecosystem and decreasing the arable land became the pressurized problems for all the producers of the present world. Moreover organic agriculture optimizes the application of biocomposts to improve soil fertility and productivity while minimizing the environmental implications and also it is necessary for sustainable agriculture. The study was conducted at St Josephs College for Women (A), Visakhapatnam Andhra Pradesh. Agro-industrial wastes of Paddy and Coffee husk were collected and were used for the biocomposting process using *Pleurotus eous*, *P. florida*, *Trichoderma asperelloides* and *Eisenia fetida*. The vermicompost was used for the growth of black gram with 6 treatments. The plant was analyzed for root length, shoot length, number of leaves, number of nodules, fresh weight and dry weight at different stages (20, 40 and 60 DAS). On 90<sup>th</sup> day the yield parameters like number of pods/plant, number of seeds/plant, length of the pod, weight of the seeds/pod, fresh weight and dry weight of the pod were analyzed. The experimental study results revealed that the treatment 6 (Pre decomposed Coffee husk, Consortium of *Pleurotus eous*, *P. florida*, *Trichoderma asperelloides* and *Eisenia fetida*) has shown a significant increase in root length (7.9,12.2 and 16.5 cm), shoot length (11.8, 25.8 and 30.7 cm), number of leaves(19.0,37.0 and 41.0) number of nodules (10.0,30.0 and 17.0), fresh weight (2.77,7.37 and 15.39 g) and dry weight (0.52,3.67 and 5.73 g) of the plant on 20 DAS, 40 DAS and 60 DAS and the yield parameters also showed the increase in number of pods/plant (39), number of seeds/plant (5), length of the pod (5.6 cm), weight of the seeds /pod (2.384 g), fresh weight of the pod (2.95 g) and dry weight (1.195 g) on 90<sup>th</sup> Day of black gram when in comparison with the control. It is to conclude that treatment 6 can be a better manure to enhance the growth and yield of plants and also it can be a great help for the farmers.

**Key words:** Black gram, *Eisenia fetida*, Paddy and Coffee husk, *Pleurotus eous*, *Pleurotus florida*, *Trichoderma asperelloides*.

The recent developments of the urban areas climate changes in the ecosystem and decreasing of the arable land became the pressurized problems for all the producers of the present world. Ghasemi Ghehsareh *et al.* (2020). Moreover organic agriculture optimizes the application of biocomposts to improve soil fertility and productivity while minimizing the environmental implications and also it is necessary for sustainable agriculture (Masunga *et al.*, 2016; Askari *et al.*, 2020). Conversion of agricultural wastes into biocomposts will reduce the pressure of chemical fertilizers and also take the important role in conditioning the nutrients in the soil as the main source of nutrient for agricultural soil. According to Sakthivigneswari and Vijayalakshmi, (2016) the best way to save the soil is to convert the organic residues to vermicomposts and it can be used as an efficient fertilizer, it contains all the necessary enzymes, vitamins and substances that are used for the plant growth. The commonly used earthworm species for the vermicompost process are *Eisenia fetida*, *Eudrilus euginea* (Blouin *et al.*, 2019; Gupta *et al.*, 2020; Ceritoglu *et al.*, 2021). Black gram is the important pulse crop which grows throughout India for it is the major source of protein hence a lot of efforts are being put to enhance the crop (Gomathinayagam *et al.*, 2021). Among the entire legumes black gram is an important

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crop which is grown in India. And it contains 24% of protein, 60% of carbohydrate and 1.3% of fat. It is commonly called as 'urd' or 'urd bean' through its ability it fixes the atmospheric nitrogen and improves the soil fertility with its root nodules (Shekhawat *et al.*, 2018). The main aim of this present study is to evaluate the effect of paddy and coffee husk vermicompost on the growth at 20, 40 and 60 days after sowing (DAS) and yield parameters (90<sup>th</sup> Day) of black gram (*Vigna mungo* L.).

The pot culture experimental study was conducted from June to August 2019 at St. Joseph's College for Women (A), Visakhapatnam and Andhra Pradesh situated at (17°43'07"N, 83°17'13"E).

#### Collection of Agro-industrial wastes

The paddy and coffee husks were collected from nearby villages in Visakhapatnam District of Andhra Pradesh. The wastes which were collected for the study were sun-dried and preserved for further study. The collected raw samples were used for the pre-decomposition with the incorporation of *Pleurotus seous*, *Pleurotus florida* spawn and *Trichoderma asperelloides*, it is done in six pits each with the measurement of 1.5 feet in length and 4 square feet width, the composts were given the following names C1, C2, C3, C4, C5 and C6. After 30 days of the pre-decomposition process, the samples were filled in plastic trays of 50×20×20 cm for the further process of vermicompost. 15 exotic earthworms *Eisenia fetida* were added to the respective trays and left for 60 days for the composting process. This whole process was done from January to March 2019.

#### Collection of seeds

The viable seeds of black gram were collected from Tamil Nadu Agricultural University, Coimbatore. The viable seeds were used for the pot culture experiments.

#### The experimental pot cultures with the treatments

The pot culture experiments were carried out with 6 treatments respectively, all the pots were filled with the paddy and coffee husk vermicompost and 7 kg of red sandy loamy soil was used. Viable seeds were sown in each pot nearly 5 plants were maintained for the study in all the treatments triplicates were maintained. The following treatments are used for the study.

#### Statistical analysis

The obtained results on 20, 40 and 60 DAS for vegetative parameters and the yield parameters on 90 DAS were analyzed statistically using One-way and Two -way ANOVA (Sigma stat 3.1).

C	Control
T <sub>1</sub>	Pre decomposed paddy husk, <i>Pleurotus eous</i> and <i>Eisenia fetida</i> (5 t/ha).
T <sub>2</sub>	Pre decomposed paddy husk, <i>Pleurotus florida</i> and <i>Eisenia fetida</i> (5 t/ha).
T <sub>3</sub>	Pre decomposed paddy husk, Consortium of <i>Pleurotus eous</i> , <i>Pleurotus florida</i> , <i>Trichoderma asperelloides</i> and <i>Eisenia fetida</i> (5 t/ha).
T <sub>4</sub>	Pre decomposed Coffee husk, <i>Pleurotus eous</i> and <i>Eisenia fetida</i> (5 t/ha).
T <sub>5</sub>	Pre decomposed Coffee husk, <i>Pleurotus florida</i> and <i>Eisenia fetida</i> (5 t/ha).
T <sub>6</sub>	Pre decomposed Coffee husk, Consortium of <i>Pleurotus eous</i> , <i>P. florida</i> , <i>Trichoderma asperelloides</i> and <i>Eisenia fetida</i> (5 t/ha).

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Treatment 6 (Pre decomposed Coffee husk, Consortium of *Pleurotus eous*, *P. florida*, *Trichoderma asperelloides* and *Eisenia fetida*) showed the maximum growth in vegetative parameters of *Vigna mungo* as it is shown in Tables (1 and 2). Root length was observed to be increasing from 20 Days after sowing (DAS) to 60 DAS in treatment 6 (7.9, 12.2 and 16.5 cm) followed by treatment 3 (6.1, 11.3 and 15.6 cm) as it is compared to control (3.1, 7.3 and 11.6 cm). Shoot length increased gradually in the treatment 6 (11.8, 25.8 and 30.7 cm) followed by the treatment 3 (11.1, 25.2 and 29.1 cm) compared to the control (8.2, 18.6 and 24.9 cm). Similar results were correlated with Raihing and Vijayalakshmi, (2022) in black gram with the application of vegetable and fruit waste, shoot length was found to be more in treatment-8 (56.9, 62.1 and 71.5 cm) and root length in treatment 8 (14.5, 17.2 and 18.5 cm).

Number of leaves showed a great increase on 20 to 60 DAS as shown in Table 1 remarkable increase was found in treatment 6 (19.0, 37.0 and 41.0) in comparison with the other treatments and it is followed by treatment 3 (17.0, 32.0 and 39.0) and the control (10.0, 20.0 and 29.0) showed the minimum number of leaves. Similar results were also reported by Palla *et al.*, (2021) the maximum number of leaves was found to be more at 90 DAS (7.70) in brinjal when it is applied with different inorganic and organic fertilizers.

Number of nodules showed an increase in 40 DAS was found to be decreasing in its number at 60 DAS and treatment 6 (10.0, 30.0 and 17.0) showed a remarkable increase among all the other treatments and the control (4.0, 11.0 and 9.0). Similar results were found in the observations of Sentirenla Changkija and Gohain, (2018) the maximum number of nodules (14.33) were found in soybean with the application of poultry manure 6 t ha + *Rhizobium* @ 20 g Kg<sup>-1</sup> seed + Phosphatica @ 20 g Kg<sup>-1</sup> (T<sub>6</sub>).

As is shown in Table 2 maximum fresh weight was found in treatment 6 (2.77, 7.37 and 15.39 g) followed by treatment 3 (1.99, 6.16 and 15.20 g) and the minimum fresh weight was found in control (1.10, 4.01 and 10.97 g) and remarkable dry weight was recorded in treatment 6 (0.52, 3.67 and 5.73 g) and it is nearly followed by treatment 3 (0.49, 3.07 and 5.06 g) and the least dry weight was recorded in control (0.19, 2.01 and 3.07 g). Similar results were in par with the work of Silpa and Vijayalakshmi, (2022) in *Vigna unguiculata* the incorporation of bio compost raw jack fruit peel + *Pleurotus florida* + *Eudrilus euginiae* in treatment 8 showed the good results in the fresh weight (5.491 g, 9.811 g and 32.516 g) and dry weight (0.855 g, 1.489 g and 3.020 g) of the plant.

As shown in Table 3 the yield parameters of black gram recorded the highest results in the treatment 6 (Pre decomposed coffee husk, consortium of *Pleurotus eous*, *P. florida*, *Trichoderma asperelloides* and *Eisenia fetida*) and it is closely followed by the treatment 3 (Pre decomposed

**Table 1:** Impact of paddy and coffee husk as the biocompost and its effect on the vegetative parameters of *Vigna mungo* (L.) Hepper.

Treatments	Root length (cm)			Shoot length (cm)			Number of leaves			Number of nodules		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
C	3.1	7.3	11.6	8.2	18.6	24.9	10.0	20.0	29.0	4.0	11.0	9.0
T <sub>1</sub>	4.7	9.7	13.9	10.5	21.1	27.2	12.0	23.0	34.0	6.0	19.0	13.0
T <sub>2</sub>	4.2	9.5	13.4	10.2	20.8	27.8	11.0	24.0	32.0	7.0	17.0	11.0
T <sub>3</sub>	6.1	11.3	15.6	11.1	25.2	29.1	17.0	32.0	39.0	9.0	25.0	15.0
T <sub>4</sub>	4.3	8.6	12.7	10.9	19.1	27.5	13.0	22.0	30.0	5.0	18.0	10.0
T <sub>5</sub>	5.2	9.9	12.5	9.9	19.7	26.9	14.0	25.0	31.0	8.0	20.0	12.0
T <sub>6</sub>	7.9	12.2	16.5	11.8	25.8	30.7	19.0	37.0	41.0	10.0	30.0	17.0
SEd	0.01858			0.17871			0.44129			0.20840		
CD	0.03754**			0.36119**			0.89191**			0.42119**		

(p&lt;0.05)

\*\*Significant at (p&lt;0.05) DAS-Days after sowing.

C- Control, T<sub>1</sub>- Raw paddy husk pre decomposed by *Pleurotus eous*+*Eisenia fetida* (5 t/ha), T<sub>2</sub>- Raw paddy husk pre decomposed by *Pleurotus florida* + *Eisenia fetida* (5 t/ha), T<sub>3</sub>- Raw paddy husk pre decomposed by consortium of *Pleurotus eous*, *Pleurotus florida*, *Trichoderma asperelloides* + *Eisenia fetida* (5t/ha), T<sub>4</sub>- Raw Coffee husk pre decomposed by *Pleurotus eous* + *Eisenia fetida* (5 t/ha), T<sub>5</sub>- Raw coffee husk pre decomposed by *Pleurotus florida* + *Eisenia fetida* (5 t/ha), T<sub>6</sub>- Raw coffee husk pre decomposed by Consortium of *Pleurotus eous*, *P. florida*, *Trichoderma asperelloides* + *Eisenia fetida* (5 t/ha).

**Table 2:** Impact of paddy and coffee husk as the biocompost and its effect on the fresh weight and dry weight of *Vigna mungo* (L.) Hepper.

Treatments	Fresh weight (g)			Dry weight (g)		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
C	1.10	4.01	10.97	0.19	2.01	3.07
T <sub>1</sub>	1.52	5.06	12.14	0.24	2.34	4.03
T <sub>2</sub>	1.51	5.17	12.09	0.28	2.15	4.21
T <sub>3</sub>	1.99	6.16	15.20	0.49	3.07	5.06
T <sub>4</sub>	1.66	5.39	12.13	0.20	2.09	4.01
T <sub>5</sub>	1.71	6.01	13.19	0.29	2.31	4.02
T <sub>6</sub>	2.77	7.37	15.39	0.52	3.67	5.73
SEd	0.19346			0.07555		
CD (p<0.05)	0.39101**			0.15250**		

\*\*Significant at (p&lt;0.05); DAS-Days after sowing.

C- Control, T<sub>1</sub>- Raw paddy husk pre decomposed by *Pleurotus eous* + *Eisenia fetida* (5 t/ha), T<sub>2</sub>- Raw paddy husk pre decomposed by *Pleurotus florida* + *Eisenia fetida* (5 t/ha), T<sub>3</sub>- Raw paddy husk pre decomposed by consortium of *Pleurotus eous*, *Pleurotus florida*, *Trichoderma asperelloides* + *Eisenia fetida* (5 t/ha), T<sub>4</sub>- Raw coffee husk pre decomposed by *Pleurotus eous* + *Eisenia fetida* (5t/ha), T<sub>5</sub>- Raw coffee husk pre decomposed by *Pleurotus florida* + *Eisenia fetida* (5 t/ha), T<sub>6</sub>- Raw coffee husk pre decomposed by Consortium of *Pleurotus eous*, *P. florida*, *Trichoderma asperelloides* + *Eisenia fetida* (5 t/ha).

paddy husk, consortium of *Pleurotus eous*, *Pleurotus florida*, *Trichoderma asperelloides* and *Eisenia fetida*) when compared to the control and all the other treatments. Treatment 6 (39) showed the maximum number of pods/plant and it is followed by the treatment 3 (37) and the minimum number showed by control (29) as well as the length of the pod was also recorded to be high in treatment 6 (5.6 cm) followed by treatment 3 (5.1 cm) and the lowest is found in control (3.5 cm). The length of the pod was found to be high in the work of Raihing and Vijayalakshmi, (2021) in treatment 8 (13.00 cm) with the application of fruit waste, cow dung, *P. eous*, *Trichoderma asperelloides* and *Eudrilus eugeniae* (5 t/ha).

The maximum number of seeds in treatment 6 and treatment 3 was found to have the similar results (5) and the control (3) showed the lowest results, similar results were found in soybean with the application of poultry manure 6 t/ha + *Rhizobium* @ 20 g Kg<sup>-1</sup> seed Phosphatica @20 g Kg<sup>-1</sup> (T6) in the maximum number of seeds/pod (2.83) (Changkija and Gohain, (2018). The maximum increase is found in the weight of the seeds/pod in treatment 6 (2.384 g) followed by treatment 3 (2.012 g) when it is compared to other treatments and the control (1.012 g), as well as pod fresh weight and dry weight also showed the highest increase in treatment 6 (2.95 and 1.195 g) followed by treatment 3 (2.87 and 1.102 g) the lowest was found in control (1.22 and 0.670 g).

**Table 3:** Impact of paddy and coffee husk as the biocompost and its effect on the yield parameters of *Vigna mungo* (L.) hepper at 90 DAS.

Treatments	Number of pods/plant	Length of the pod (cm)	Number of seeds/pod	weight of the seeds/pod (g)	Pod fresh weight (g)	Pod dry weight (g)
C	29	3.5	3	1.012	1.22	0.670
T <sub>1</sub>	36	4.0	4	1.885	1.61	0.831
T <sub>2</sub>	34	3.9	4	1.911	1.77	0.901
T <sub>3</sub>	37	5.1	5	2.012	2.87	1.102
T <sub>4</sub>	35	4.3	5	1.117	1.75	0.811
T <sub>5</sub>	36	4.7	4	1.224	1.67	0.728
T <sub>6</sub>	39	5.6	5	2.384	2.95	1.195
SEd	0.0440	0.3171	0.1688	0.2535	0.1936	0.0402
CD (p<0.05)	0.0943**	0.6802**	0.3622**	0.5438**	0.4153**	0.0863**

\*\*Significant at (p<0.05) DAS-Days after sowing.

C-Control, T<sub>1</sub>- Raw paddy husk pre decomposed by *Pleurotus eous* + *Eisenia fetida* (5 t/ha), T<sub>2</sub>- Raw paddy husk pre decomposed by *Pleurotus florida* + *Eisenia fetida* (5 t/ha), T<sub>3</sub>- Raw paddy husk pre decomposed by consortium of *Pleurotus eous*, *Pleurotus florida*, *Trichoderma asperelloides* + *Eisenia fetida* (5 t/ha), T<sub>4</sub>- Raw Coffee husk pre decomposed by *Pleurotus eous* + *Eisenia fetida* (5 t/ha), T<sub>5</sub>- Raw coffee husk pre decomposed by *Pleurotus florida* + *Eisenia fetida* (5 t/ha), T<sub>6</sub>- Raw Coffee husk pre decomposed by Consortium of *Pleurotus eous*, *P. florida*, *Trichoderma asperelloides* + *Eisenia fetida* (5 t/ha).

Similar results are correlated with Kumar *et al.* (2021) who found the increase in the yield of pea crops 40 to 61% and capsicum crop 14-48% with the application of biomass with matkakhad, rock phosphate, gypsum, patent Kali and lime @ 1% and with the inclusion of *Trichoderma*, *Rhizobium*, *Azotobacter* along with PSB. Veeral and Kalaimathi, (2021) observed similar results in groundnut plants an increase in the plant height (29.9 cm) in the treatment 7 (Press mud @ 12.5 t/ha+50% RDF and *Rhizobia* @ 2 kg ha) and the number of pods/plant (27.4). Significant results were found in cluster bean with the application of chemical fertilizer with *rhizobium* (2.5 g). Treatment 16 showed the highest number of pods (221.2) (Gul *et al.*, 2019). The maximum number of pods/plant (45.94) were found in soya beans with the application of 90 P<sub>2</sub>O<sub>5</sub> level (kg/ha) (Edwin Luikham *et al.*, 2018).

## CONCLUSION

The application of Agro-industrial wastes Paddy and Coffee husk biocompost on black gram and its influence on the growth and yield parameters of black gram was proved with maximum results in treatment 6 (T<sub>6</sub>). The study concludes that it can be a substitute for the chemicals and it can be used as promising, sustainable, safe and eco-friendly manure to have the best growth, yield and productivity of plants.

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## Conflict of interest

I declare that Authors have no conflict of interest regarding this article.

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