3

Materials and Methods

In this chapter, the details of methodology regarding enrichment of biocompost, composting technology, vermicomposting, evaluation of compost maturity, pot culture experiments, incorporation of different composted wastes treatments on the biometric characters, biochemical parameters, yield, leghaemoglobin content in root nodules, soil analysis, antibacterial and antioxidant profile and statistical analysis of the data are presented here.

PHASE I

3.1 Collection of waste

The vegetable waste is collected from in and around Saibaba Colony, Coimbatore, Tamil Nadu. The fruits waste is collected from outside and inside the campus fruit stall of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore (11.0196° N, 76.9504° E) in large quantity which is cut into small pieces, sun dried and stored in polythene bag.

Collection of Earthworm

The *Eudrilus eugeniae* Kinberg earthworm species was collected from ICAR KVK (Krishi Vigyan Kendra) Coimbatore, Tamil Nadu.

3.1.1 Composting procedure

Compost pit Preparation

For composting, pits measuring 1.5 feet length and 4 square feet width was dug. Eight composting pits namely Compost 1, 2, 3 and 4 were used for vegetable waste composting whereas Compost 5, 6, 7 and 8 for fruit waste composting.

Winter compost preparation

The composting was carried out during winter season (November-January) for 90 days with both vegetable and fruit waste which is subjected to decomposition by introducing fungus *Pleurotus eous* (APK1) and *Trichoderma asperelloides*, cow dung and earthworm species to achieve an efficient and good quality manure.

PLATE – 3 COLLECTION OF VEGETABLE WASTE

PLATE – 4 COLLECTION OF FRUIT WASTE

Compost 1

1kg of vegetable waste was transferred to the C_1 pit and 500gm cow dung was added above the sample. The same step was followed until the heap reached the height of above 1 meter. The moisture content of the pit was maintained by sprinkling of water at regular interval. Turning along the heap was done manually every week during the process of composting.

Compost 2

In this pit, about 1kg of vegetable waste was filled with 20g of *Pleurotus eous* (APK1) spawn and cow dung was uniformly spread until the heap reaches the height of above 1 meter. The water content in the pit was maintained by spraying at regular interval. Manual turning of the heap was carried out every week to accelerate the decomposition process.

Compost 3

1kg of compost along with 500gm cow dung and 20g *Trichoderma asperelloides* spawn was spread above one after another till the compost reaches the height of 1 meter uniformly. Turning the heap and sprinkling of water was done every week.

Compost 4

In this pit, 1kg of vegetable waste and 500gm cow dung along with 20g of consortium of fungus (*Pleurotus eous* (APK1) + *Trichoderma asperelloides*) was filled. The same process was followed until it reaches the height of 1 meter. Watering the heap was done every week and turning of the compost heap was carried out to accelerate the decomposition process.

Compost 5

The sundried 1kg of fruit waste along with 500gm cow dung was transferred and filled in this pit. The same process of filling the pit with fruit waste and cow dung was followed till the heap reaches 1-meter height. Manual turning and sprinkling of water was done every week during composting process.

Compost 6

1kg of fruit waste, 500gm cow dung and 20g *Pleurotus eous* (APK1) was spread one after another until the heap reaches 1 meter. The moisture content was maintained by

spraying the water in the pit. To stimulate the decomposition process, manual turning of the heap was done every week.

Compost 7

In this pit, 1kg of fruit waste, 20g of *Trichoderma asperelloides* and 500gm cow dung was filled and spread uniformly. The moisture content in the heap was maintained at about 60-70% by sprinkling water. Turning was done manually every week to accelerate the decomposition process.

Compost 8

The pit was filled with 1kg fruit waste along with 500gm cow dung and 20g consortium of fungus (*Pleurotus eous* (APK1) + *Trichoderma asperelloides*) spawn was spread one after another uniformly until it reaches the height of 1 meter. To maintain the moisture content in the heap, manual sprinkling of water was done. To accelerate the decomposition process, turning of the heap was carried out every week during composting period.

Summer compost preparation

Above same method was repeated for all the compost treatments but the composting process was carried out during summer season (May-July) respectively.

The compost was allowed to decompose or degrade the waste sample in the pit for 30 days. Vermicomposting technology was adopted after removing and transferring the compost to the tub after 30 days.

3.1.2 Experimental vermicompost tray preparation

Plastic tray of about 60 × 30 × 15 cm was purchased from a local departmental store and made a small hole at the bottom of the tray to facilitate the draining of excessive water content in the tube. Vermicompost bed was arranged in a tray at 2:1 ratio (2kg of pre-decomposed vegetable or fruit waste : 1kg of cow dung). The moisture content of the substrate was maintained at 60-80% and kept for 24-hour for stabilization. Twenty-five healthy exotic earthworm species (*Eudrilus eugeniae* Kinberg) were inoculated into their respective experimental tray. To maintain the moisture content, water is sprayed regularly and kept in shady place undisturbed for 60 days. At the end of composting, the earthworm was separated from the compost by sieving and obtained a dark colour compost.

PLATE - 5

Biocomposting of Vegetable Waste

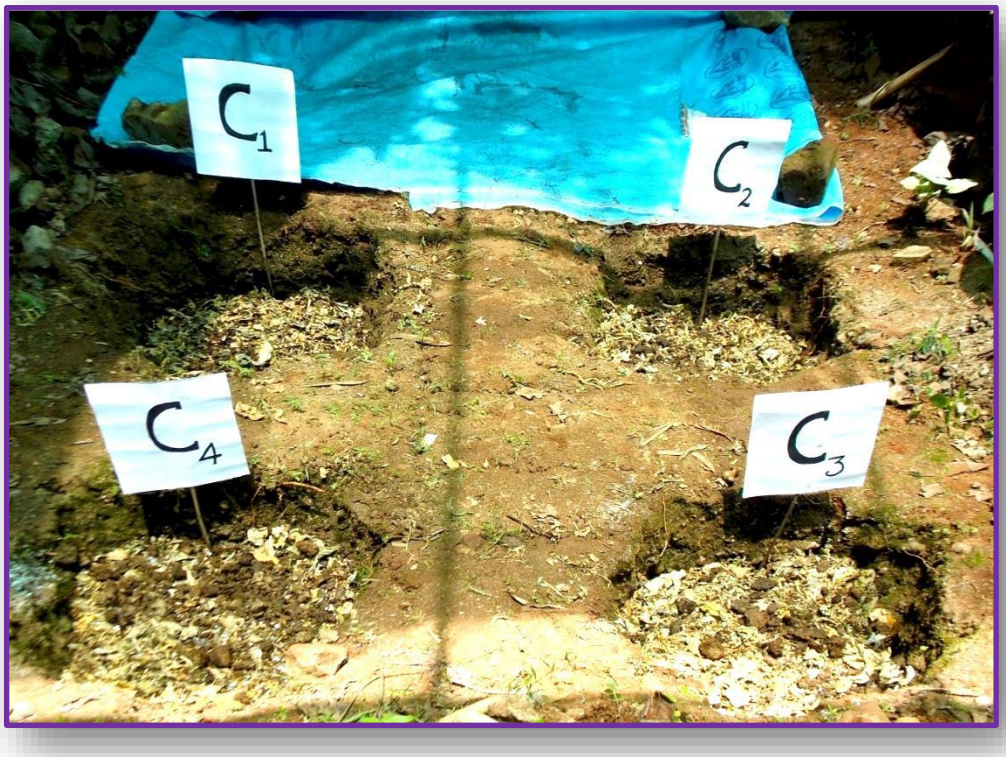
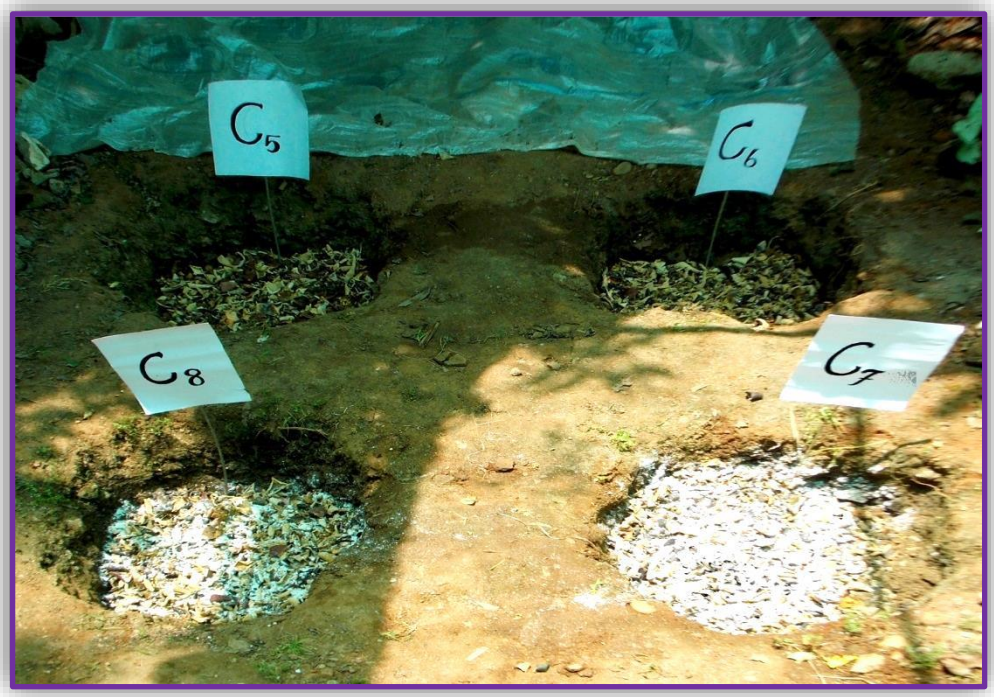


PLATE – 6

Biocomposting of Fruit Waste



3.2 Microbial population during composting

Enumeration of bacteria, fungi and actinomycetes was carried out by taking one gram of each sample in sterile conical flasks containing 9ml of distilled water, shaken for 30 min in vortex mixer and used as stock from which various dilutions were prepared ranging from 10^1 to 10^7 with sterile distilled water as described by Kannan (1996). One ml each of the dilutions of 10^7 (bacteria), 10^4 (fungi) and 10^2 (actinomycetes) from each sample was transferred to sterile petri plates containing nutrient agar medium for bacteria, potato dextrose agar medium fot fungi and Ken-Knights agar medium for actinomycetes in triplicates and incubated for one day, three days and seven days respectively. Microbial colonies were counted at regular intervals of 30 days, 60 days and 90 days during decomposition period. With the use of a colony counter, the number of viable colonies was calculated.

3.2.1 Evaluation of compost maturity

Physical and chemical assays of raw and composted vegetable and fruit waste were analysed using standard method. These techniques have been put forth for determining maturity levels.

Physical parameters

The pH and Electric conductivity of raw and composted wastes were analysed by using a glass electrode and electrical conductivity bridge

Chemical parameters

1. Lignin (Goering and Vansoest, 1975) Appendix – 1
2. Cellulose (Updegroff, 1969) Appendix – 2
3. Organic carbon (%) (Walkley and Black, 1934) Appendix – 3
4. Total Nitrogen (%) (Humphries, 1956) Appendix – 4
5. Total Phosphorus (%) (Jackson, 1973) Appendix – 5
6. Total Potassium (%) (Jackson, 1973) Appendix – 6
7. Calcium and magnesium (%) (Jackson, 1973) Appendix – 7
8. C:N ratio

3.2.2 Fourier Transform-infrared (FT-IR) spectrum analysis

In order to determine the possible functional group during degradation process, FT-IR analysis was carried out. Test sample and control sample were blended independently with KBr to obtain pellets. Vermicompost samples weighing two gram each were mixed with 200 mg of KBr (spectroscopy grade), homogenised, and pelletized for ten minutes under vacuum.

Readings were recorded at a frequency of 0.5 cm/s and a range of 4000-400 cm^{-1} using Perkin – Elmer 2000 FT-IR spectrophotometer.

PHASE II

3.3 Pot culture experiment

A pot culture experiment was carried out in black gram [*Vigna mungo* (L.) Hepper] Var. Co 6 and Lablab [*Lablab purpureus* (L.) Sweet] Var. Co (Gb) 14 grown in compost treatments carried out in winter (November-January) and summer (May-July) season as test crops to evaluate the effect of vegetable and fruit waste biocompost.

Collection of seeds

The seeds of black gram [*Vigna mungo* (L.) Hepper] Var. Co 6 and Lablab [*Lablab purpureus* (L.) Sweet] Var. Co (Gb) 14 was collected from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The plant specimens were authenticated by M. U. Sharief, Scientist 'F' & Head of Office, Botanical Survey of India, TNAU campus, Coimbatore-641003, with voucher specimen number BSI/SRC/5/23/2022/Tech./401 (Annexure-I) and BSI/SRC/5/23/2022/Tech./189 (Annexure-II)

3.3.1 Treatment details

The experiment consists of eight treatments of vegetable and fruit waste biocompost. The different treatments of pot culture were as follows

C - Control

T_1 - Vegetable wastes (V.W) + cow dung + *Eudrilus eugeniae* (5 t/ha)

T_2 - Vegetable wastes (V.W) + cow dung + *Pleurotus eous* + *Eudrilus eugeniae* (5 t/ha)

T_3 -Vegetable wastes (V.W) + cow dung + *Trichoderma asperelloides* + *Eudrilus eugeniae*(5 t/ha)

T_4 -Vegetable wastes (V.W) + cow dung + *Pleurotus eous* + *Trichoderma asperelloides* + *Eudrilus eugeniae* (5 t/ha)

T_5 - Fruit wastes (F.W) + cow dung + *Eudrilus eugeniae* (5 t/ha)

T_6 - Fruit wastes (F.W) + cow dung + *Pleurotus eous* + *Eudrilus eugeniae* (5 t/ha)

T_7 - Fruit wastes (F.W) + cow dung + *Trichoderma asperelloides* + *Eudrilus eugeniae* (5 t/ha)

T_8 - Fruit wastes (F.W) + cow dung + *Pleurotus eous* + *Trichoderma asperelloides* + *Eudrilus eugeniae* (5 t/ha)

Biometric, biochemical, yield parameters and leghaemoglobin content in root nodules were recorded in all the treatments of the test crops. Antioxidant and antibacterial activity was conducted on the best treatment and control treatment of the test crops.

3.3.2 Treatment application and cultivation

The soil selected for the study is sandy clay loam soil. Stone and pebbles from the soil were removed from the soil before filling the pot and all the pots were filled with 7kg of selected soil. Vegetable and fruit biocompost were applied to their respective pots and thoroughly mixed well. About 10-12 viable seeds of black gram and lablab were sown in each pot with three replications. Around 6-7 healthy plant were maintained in the pot after germination. As per recommendation from Tamil Nadu Agricultural University, Coimbatore, plant protection measures and other cultural practices were followed.

3.4 Biometric parameters

The plant black gram and lablab was uprooted from each treatment on 15 DAS, 35 DAS and 55 DAS to evaluate the following vegetative parameters

1. Root length (cm)
2. Shoot length (cm)
3. Number of leaves
4. Number of nodules
5. Number of flowers/plant
6. Plant fresh weight (gm)
7. Plant dry weight (gm)

3.5 Yield parameters

On the 75th day, the plants were uprooted from the respective pots and the following yield parameters were observed

1. Number of pods /plant
2. Length of pod (cm)
3. Number of seeds /pod
4. Weight of seeds/pod (g)
5. Pod fresh weight (g)
6. Pod dry weight (g)

PHASE III

3.6 Biochemical Analysis

Biochemical parameters were analysed in leaves on 15 DAS, 35 DAS, 55 DAS and seed on 75 DAS of black gram and lablab.

3.6.1 Protein

Estimation of protein (Lowry *et al*., 1951)

- APPENDIX- 8

3.6.2 Carbohydrate

Estimation of carbohydrate (Hedge and Hofreiter, 1962)

Anthrone method- APPENDIX- 9

3.6.3 Chlorophyll

Estimation of chlorophyll (Arnon, 1949)

- APPENDIX- 10

3.6.4 Crude protein

Estimation of crude protein content (A.O.A.C., 2016)

- APPENDIX- 11

3.7 Analysis of Leghaemoglobin

Estimation of leghaemoglobin content (Appleby and Bergersen, 1980)

APPENDIX – 12

PHASE IV

3.8 Experimental Pre and Post harvest Soil Sample Analysis

1. Available nitrogen (Subbiah and Asija, 1956)

Alkaline permanganate method - APPENDIX- 13

2. Available phosphorus (Jackson, 1973)

Colorimetry method - APPENDIX- 14

3. Available potassium (Standford and English, 1949)

Flame photometry - APPENDIX- 15

3.9 Antioxidant Activity

3.9.1 Processing of plant seed materials

The best treatment T_8 – Fruit waste (F.W.) + cow dung + *Pleurotus eous* + *Trichoderma asperelloides* + *Eudrilus eugeniae* (5 t/ha) grown plant seed was collected and dried under shade. The dried seed sample were powdered and packed in polythene bag for further study.

3.9.2 Successive solvent extraction

The dried seed powdered of selected plant were extracted using Soxhlet apparatus successively with methanol and aqueous. Each time before extracting with the next solvent, the material was dried in hot air oven. Finally, the material was macerated using hot water with occasional stirring for 24 h and the aqueous extract was filtered. The different solvent extracts were concentrated by rotary evaporator and then air dried. The extracts were stored in desiccators until further analysis

3.9.3 Hydrogen peroxide scavenging activity (Ruch *et al*., 1989)

The ability of the extract to scavenge hydrogen peroxide was determined by method of Ruch *et al*., (1989) (APPENDIX- 16)

3.9.4 Reducing power assay

The Reducing power assay was determined by Oyaizu, (1986) in methanol and aqueous seed extract of black gram grown in winter and summer compost (APPENDIX- 17).

3.9.5 Nitric oxide radical scavenging activity

The ability of the methanol and aqueous seed extract of black grown in winter and summer compost that exhibit nitric oxide was determined by Green *et al*., (1982) method (APPENDIX- 18)

3.9.6 DPPH radical scavenging activity

The ability of Black gram and Lablab methanol seed extract of best treatment and Control treatment was measured according to the procedure of Mensor *et al*., (2001) (APPENDIX- 19)

3.10 Antibacterial activity

Bacterial Strains used: -

The bacterial strains *Escherichia coli* and *Staphylococcus aureus* were selected to investigate the antibacterial activity of the black gram and lablab plants grown in winter and summer season compost.

3.10.2 Assessment of antibacterial activity (well diffusion method)

After the characterization of selected microbes, the antibacterial activity on the selected plant seeds was carried out using agar well diffusion method according to the procedure of Bauer *et al.,* (1996) (APPENDIX- 20) by measuring the diameter of zone of

inhibition around the well. The antibacterial activity was assessed in the petri plate using muller hinton agar medium.

3.11 Statistical Analysis

The data obtained on biometric observations (root length, shoot length, number of leaves, number of nodules, number of flowers/plant, fresh weight and dry weight.), biochemical analysis (15, 35 and 55 DAS) and leghaemoglobin content on 25 and 50 DAS, yield parameters (number of pods/plant, length of pod, weight of pod, number of seeds per pod, weight of seeds per pod, pod fresh weight and dry weight) on 75 DAS, antioxidant and antibacterial activity for the seed extract of the selected best treatment and control were subjected to the statistical analysis (one way and two way ANOVA).