3

MATERIALS AND METHODS

The details regarding biodegradation of groundnut shell and toddy palm shell by the microbial consortium, *Trichoderma asperelloides* and *Eisenia fetida* to assess the efficacy of various treatments, field culture studies, biometric, biochemical, yield parameters and soil fertility status of selected test crops were recorded and the statistical evaluation of the data are presented in this chapter.

PHASE I

3.1 Composting

3.1.1 Collection of materials

The agricultural residue groundnut shell was obtained from groundnut field, Pollachi and toddy palm shell was collected from roadside litter zones and toddy palm vendor shops in and around the city of Coimbatore, Tamil Nadu. The collected groundnut shell and toddy palm shell were incised into small pieces and kept under sunlight for one week to dry till the complete removal of moisture contents.

3.1.2 Collection of microorganisms and earthworms

Paecilomyces variotti (MTCC-6581), *Bacillus licheniformis* (MTCC-10498), *Pleurotus florida* (MTCC-9194) and *Streptomyces lavendulae* (MTCC-6821) were collected from the Institute of Microbial Technology, Chandigarh and *Trichoderma asperelloides* was collected at Tamil Nadu Agricultural University, Coimbatore. The tiger worm *Eisenia fetida* was collected from Selvam Eyarkai Angadi at Udumalpet.

3.1.3 Preparation of microbial consortium

The sub-cultured microorganisms such as bacteria (*Bacillus licheniformis*), cellulolytic fungi (*Paecilomyces variotti*), lignolytic fungi (*Pleurotus florida*) and actinobacteria (*Streptomyces lavendulae*) were cultured from the mother sources and around 50 ml of culture was inoculated into 500 ml of respective culture medium. Potato dextrose agar medium for fungi, Nutrient agar medium for bacteria and Kenknights &

Munaier's medium for actinobacteria incubated at 25℃, 35℃ and 30℃ for 3 days, 1 day and 7 days respectively. Further, an equal volume of fungi, bacteria and actinobacteria in the ratio of 1:1:1, approximately 200 g of cultures were mixed with cow dung slurry in the ratio of 2 kg of cow dung in 20 L of water kept at room temperature for one day.

TREATMENTS	COMPOSITION OF COMPOST
C1	Groundnut shell + *Trichoderma asperelloides* + *Eisenia fetida*
C2	Groundnut shell + Microbial consortium
C3	Groundnut shell + Microbial consortium + *Eisenia fetida*
C4	Toddy palm shell + *Trichoderma asperelloides* + *Eisenia fetida*
C5	Toddy palm shell + Microbial consortium
C6	Toddy palm shell + Microbial consortium + *Eisenia fetida*

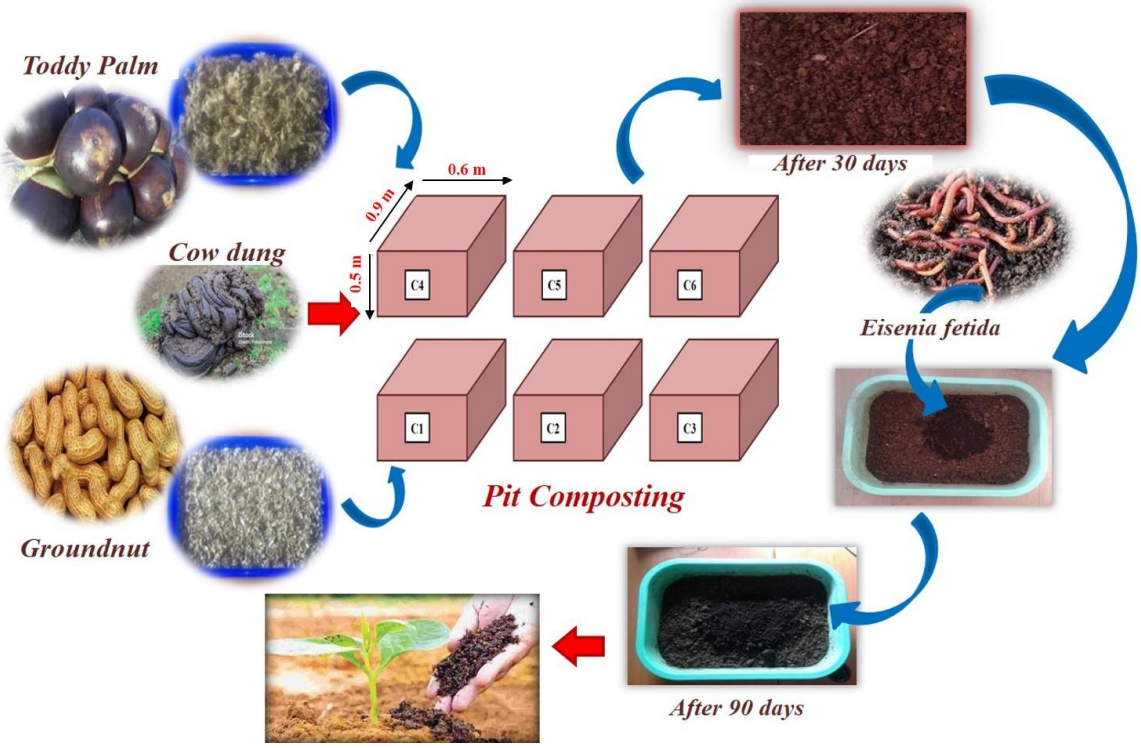
3.1.4 Process of composting

Composting was performed in pit method at Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India located at 76.9504° E and 11.0196° N. The study consists of six composting pits named C_1, C_2, C_3, C_4, C_5 and C_6 (C standard for compost) and each pit measured at 0.9m length, 0.6m width and 0.5m depth. 20 kg of dried groundnut shell was transferred into C_1, C_2, C_3 and toddy palm shell was transferred into C_4, C_5 and C_6 along with animal manure (cow dung). Mostly, animal manure is used for vermicomposting which accelerate the decomposition process and are suitable for earthworm breeding. Addition of 80 g of *Trichoderma asperelloides* into C_1, C_4 and the mixture of about 400 g of the consortium of microorganisms and cow dung slurry was sprayed into C_2, C_3, C_5 and C_6 homogeneously spread over like a sandwich. Sprinkled water to prevent drying and it was thoroughly mixed once a week. After one month the groundnut shell and toddy palm shell were partially decomposed which is suitable for earthworm feeding. C_1, C_3, C_4 and C_6 composts were transferred into the plastic trays measuring 50×35×15cm (length, width and depth). Holes were made at the bottom of the tray to drain the excess amount of water. Healthy epigeic earthworm (*Eisenia fetida*) was inoculated into the plastic trays containing respective samples. The trays were kept undisturbed in a shaded area for 60 days, maintaining a

moisture level of around 75%. At the end of the composting, it turns dark brown to a black color as shown in the Plate 6. The earthworms were alive after composting and separated by sieving.

Plate – 6

Preparation of Groundnut shell and Toddy palm shell Biocompost



3.1.5 Enumeration of microbial populations

9 ml of distilled water was taken in a sterile conical flask containing 1 gram of compost sample, continuously shaken for 30 minutes with the help of a vortex mixer. 1 ml of 10^6 and 10^4 serial dilutions of the samples were taken with the support of sterile pipettes and inoculated into sterile petri dishes containing potato dextrose agar medium for fungi, nutrient agar medium for bacteria and kenknights & munaier's medium for actinobacteria incubated at three days, one day and seven days respectively. Afterwards, the viable colonies were counted during the decomposition of the groundnut shell and toddy palm shell at regular intervals of 30^{th}, 60^{th} and 90^{th} days with the help of a colony counter.

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

3.1.6 Evaluation of compost maturity

Physical and chemical parameters of bio-composted groundnut shell and toddy palm shell were analyzed based on the following method proposed for estimating the degree of maturity.

3.1.7 Physical parameters

pH and electrical conductivity were determined by pH meter and conductivity bridge in the ratio of sample and water (1:10 w/v) respectively.

CHEMICAL PARAMETERS		
Lignin (%)	Goering and Van Soest, 1975	Appendix – 1
Cellulose (%)	Updegroff, 1969	Appendix – 2
Organic carbon (%)	Walkley and Black, 1934	Appendix – 3
Total nitrogen (%)	Humphries, 1956	Appendix – 4
Total phosphorus (%)	Jackson, 1973	Appendix – 5
Total potassium (%)		Appendix – 6
Calcium and magnesium (%)		Appendix - 7

3.1.8 Fourier transform infrared spectroscopy

FT-IR analysis samples of raw and composted groundnut shell and toddy palm shell were carried out. The samples were dried to avoid moisture content interruption. Dried homogeneous samples of 2 mg was mixed with 400 mg of Potassium Bromide and compressed as pellets. Transmittance responses were recorded from 4000 to 400 cm^{-1} on a Shimadzu FT-IR 8400S spectrometer. The FT-IR spectra of groundnut shell and toddy palm shell were compared with their processed composts (C_1, C_2 & C_3) and (C_4, C_5 & C_6) respectively.

3.1.9 X-ray diffraction method

XRD analysis of raw groundnut shell, toddy palm shell and composted samples (C_3 & C_6) were used to ensure the degradation level by chemical and physical structural changes. The same dried samples used for FT-IR were powdered well and used for XRD analysis. The samples were prepared by grinding them into fine powder and analyzed by using Empyrean nano edition.

3.1.10 Scanning electron microscopy

Scanning Electron Microscopy (Carl Zeiss EVO 18) was used for morphological studies. The prepared fine compost powder was dried at room temperature and used for SEM analysis. Nonporous carbon tape was used to carry the sample for analysis.

PHASE II

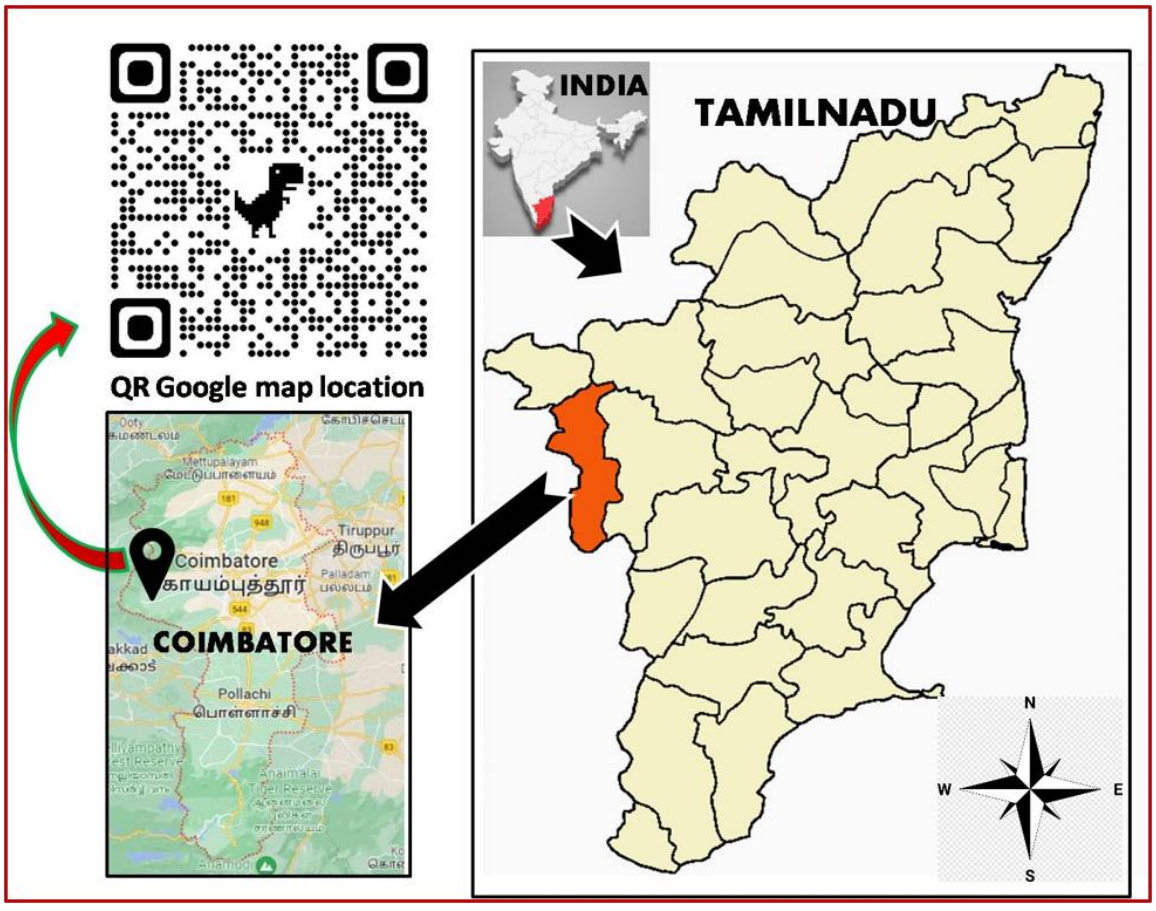
3.2 Field studies

3.2.1 Geographical situation of Experimental site

A field experiment was conducted during the period of January - April 2019 at Aalandhurai, Coimbatore, Tamil Nadu located at 11°1'6" North latitude and 76°58'21" East longitude of India shown in Plate 7.

Plate - 7

Area of Study



3.2.2 Climate and soil condition

The average annual rainfall is 952 mm and the temperature ranges from 27.8℃ to 29.6℃. The soil type of the experimental field was red clay loam soil with 5.3 pH.

3.2.3 Field preparation

The experimental plot was ploughed repeatedly by tractor to fine tilth. Ridges and furrows was opened at 30 cm distance. Field was divided into small plots about 4.6 m length and 1.4 m width according to the treatments and three replications with randomized block design. About 5 t/ha of prepared compost was incorporated into the soil depending on the treatments (Plate 8).

Plate – 8

Details of Experimental Field Preparation

A – Ridges and furrows was opened, **B** – Inoculation of compost, **C** – Drip irrigation, **D** – Emergence of new plants as shown in the plate **E** to **H** (Bhendi, Cluster bean, Coriander and Fenugreek)

3.2.4 Collection of seeds

Bhendi (CO 4), Cluster bean (MDU 1), Coriander (Co (CR) 4) and Fenugreek (Co 2) seeds were purchased from Tamil Nadu Agricultural University, Coimbatore.

3.2.5 Authentication of Test Crops

The plant samples, Bhendi – *Abelmoschus esculentus* (L.) Moench (Identification No: BSI/SRC/5/23/2023-24/Tech/451) Annexure - 1, Cluster Bean – *Cyamopsis tetragonoloba* (L.) Taub (Identification No BSI/SRC/5/23/2023-24/Tech/452) Annexure – 2, Coriander – *Coriandrum sativum* L. (Identification No: BSI/SRC/5/23/2023-24/Tech/450) Annexure - 3 and Fenugreek – *Trigonella foenum-graecum* L. (Identification No: BSI/SRC/5/23/2023-24/Tech/453) Annexure – 4, has been authenticated by Dr. M. U Sharief, Scientist F & Head of office, Botanical Survey of India, Tamil Nadu.

DETAILS OF TREATMENTS	
C	Control (without compost)
T_1	Groundnut shell + *Trichoderma asperelloides* + *Eisenia fetida* (5 t/ha)
T_2	Groundnut shell + Microbial consortium (5 t/ha)
T_3	Groundnut shell + Microbial consortium + *Eisenia fetida* (5 t/ha)
T_4	Toddy palm shell + *Trichoderma asperelloides* + *Eisenia fetida* (5 t/ha)
T_5	Toddy palm shell + Microbial consortium (5 t/ha)
T_6	Toddy palm shell + Microbial consortium + *Eisenia fetida* (5 t/ha)

3.2.6 Application of treatment and cultivation

Seeds were soaked in the water one hour before sowing and were sown at the depth of 2 cm. Bhendi and cluster bean seeds were sown in zig zag manner at a distance of 30 cm and coriander and fenugreek seeds were sown at a distance of 15 cm. Necessary amount of water was supplied by drip irrigation at regular intervals of two days and weeds was removed by hand weeding once in ten days.

3.2.7 Growth parameters

On 25, 50 and 75 DAS (bhendi, cluster bean, coriander and fenugreek) plants were uprooted from the field and the following vegetative characters were noted. Root length

(cm), Shoot length (cm), Number of leaves, Number of branches, Diameter of leaf (cm), Number of flowers, Number of umbels, Number of nodules, Fresh weight of plant (g) and Dry weight plant (g) respectively.

3.2.8 Yield parameters

On 90 DAS the yield characters like Number of fruits/plant, Number of pods/plant, Length of fruit (cm), Length of pod (cm), Diameter of fruit (cm), Number of seeds/plant, Yield/plant (g), Yield/plot (kg), Yield/hectare (kg), Straw yield/hectare (kg), Fresh weight of fruit (g), Fresh weight of pod (g), Dry weight of fruit (g) and Dry weight of pod (g) in selected test crops respectively.

PHASE III

3.3 Biochemical analysis

The protein, carbohydrate, chlorophyll a, chlorophyll b and total chlorophyll content in leaves of bhendi, cluster bean, coriander and fenugreek were analyzed on 25, 50 and 75 DAS and the leghaemoglobin content in root nodules of cluster bean and fenugreek were studied at 25, 50 and 75 days after sowing. Yield carbohydrate and protein content in fruit of bhendi, pods of cluster bean and seed of coriander and fenugreek were analyzed on 90 DAS of plant growth.

BIOCHEMICAL ANALYSIS		
Carbohydrate content	(Hedge and Hofreiter, 1962)	Appendix - 8
Protein content	(Lowry *et al*., 1951)	Appendix - 9
Chlorophyll content	(Arnon, 1949)	Appendix - 10
leghaemoglobin content	(Appleby and Bergersen, 1980)	Appendix - 11

3.4 Soil nutrients status

The soil samples were collected from the experimental plots at three different stages at 20 cm depth. Before and after compost application and post-harvested soil samples were analysed by the following methods.

3.4.1 pH and electrical conductivity

One gram of soil was mixed with 10 ml of distilled water and recorded with the help of pH meter and conductivity bridge.

SOIL ANALYSIS		
Available nitrogen (Kg/ha)	(Subbiah and Asija, 1956)	Appendix - 12
Available phosphorus (Kg/ha)	(Jackson, 1973)	Appendix - 13
Available potassium (Kg/ha)	(Standford and English, 1949)	Appendix - 14

PHASE IV

3.5 Best treatment

Based on the growth, yield and biochemical analysis of field studies the T_6 (Toddy palm shell + Microbial consortium + *Eisenia fetida*) gave the best results and the best treatment sample was compared with effective microorganisms and coir pith. The study was conducted during the period of January - April 2020 at Aalandhurai, Coimbatore.

3.5.1 Preparation of effective microorganisms

An equal amount (250 g) of pumpkin, papaya, banana and jaggery were crushed and mixed with one litre of distilled water and the mixture was transferred into an airtight container and kept in dark place for one month. At regular interval of three days the gas was released by loosening the bottle cap. After one month the mixture was fully fermented and appeared sweet sour smell which was diluted with water in the ratio of 10 ml of solution in 1 litre of water.

3.5.2 Coir pith

Coir pith was brought from Balaji nursery, Coimbatore and it was soaked in the water for 30 minutes before use. Coir pith and compost (T6) were applied before sowing the seed and effective microorganisms was sprayed after thinning the plant.

3.5.3 Growth parameters

Root length (cm), shoot length (cm), number of leaves, diameter of leaf (cm), number of flowers, number of umbels, number of nodules, fresh weight of plant (g) and dry weight of plant (g) were analyzed on 25, 50 and 75 DAS.

TREATMENT APPLICATION	
C	Control
T_1	Coir pith (5t ha^{-1})
T_2	Best treatment (Toddy palm shell + Microbial consortium + *Eisenia fetida*) (5t ha^{-1})
T_3	Effective microorganisms (1L/m^2)

3.5.4 Yield parameters

Number of fruits and pods, length of fruit and pod (cm), diameter of fruit (cm), number of seeds, yield per plant (g), yield per plot (kg), yield per hectare (kg), straw yield per hectare (kg), fresh weight of fruit and pod (g) and dry weight of fruit and pod (g) were analyzed on 90 DAS.

3.5.5 Biochemical characters

The protein (Lowry *et al*., 1951) Appendix - 9, carbohydrate (Hedge and Hofreiter, 1962) Appendix - 8, chlorophyll content (Arnon, 1949) Appendix – 10 and leghaemoglobin content (Appleby and Bergersen, 1980) Appendix – 11 were studied on 25, 50, 75 and 90 DAS.

3.5.6 Soil status

The pH, electrical conductivity, Available nitrogen (Subbiah and Asija, 1956) Appendix - 12, Available phosphorus (Jackson, 1973) Appendix – 13 and Available potassium (Standford and English, 1949) Appendix – 14 were analyzed before and after compost application and post-harvested soil.

3.6 Statistical Analysis

In the present study, the experimental findings are provided in the form of graphs (Figure) and table with the help of Microsoft Excel (Version 2019) and Origin (Version Pro 8.5) software. The data obtained from the microbial population during composting, as well as various vegetative growth and biochemical characteristics of selected plants were statistically analyzed by two-way and yield parameters were statistically analyzed by one-way ANOVA using SPSS (Version Sigma Stat 3.1). The values are given in the respective tables and figures (graphs) in the chapter 4 and the based on the results conclusion were drawn.