3

MATERIALS AND METHODS

The methods concerning the decomposition of the agro wastes sugarcane trash and sugarcane bagasse. Various biocomposting treatments, pit composting, vermicomposting, total microbial load, physico-chemical characteristic, evaluation of compost maturity, field culture experiment, application of compost into various treatments and evaluation of biometric, biochemical content and yield parameters of onion (*Allium cepa* L.), black nightshade (*Solanum nigrum* L.), tomato (*Solanum lycopersicum* L.) and brinjal (*Solanum melongena* L.). Pre and post-harvest soil status, soil microbial population, antioxidant activity, antibacterial activity and statistical representation of the data were presented in this chapter.

PHASE 1

3.1 COLLECTION OF AGRO-INDUSTRIAL WASTE

PLATE 7

AGRO-INDUSTRIAL WASTE

The agro industrial wastes of sugarcane trash and sugarcane bagasse were collected in large amounts from sugarcane fields situated in Thadakam, Coimbatore. The collected waste was chopped into small pieces. It was sun dried and stored in bags. (Plate 7)

Collection of Microorganisms

Spawn was collected from Tamil Nadu Agricultural University (TNAU) Coimbatore. The microorganisms *Pleurotus florida* and *Trichoderma asperelloides*. Earthworm *Eudrilus eugeniae* Kinberg were obtained from KVK (Krishi Vigyan Kendra) Coimbatore.

3.1.1 Preparation of Microbial Consortium

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

3.1.2 Composting Procedure

Compost Pit Preparation

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

Sugarcane Trash Compost

Compost 1 (C_1) The sundried 2kg of sugarcane trash agro-waste was transferred to C_1 pit, spread with 20g of *Pleurotus florida* spawn uniformly and sandwiched above with a layer of sugarcane trash waste. This process was repeated till the heap reaches a height of above 1 meter. The moisture content was maintained by sprinkling water at regular interval. Turning the agro-waste was manually done every week during composting process. After 30 days vermicomposting process was adopted.

Compost 2 (C_2) pit was filled with 2 kg of sugarcane trash waste along with 20g of *Trichoderma asperelloides*. This process was repeated till the heap reached a height of above one meter. To accelerate the decomposition process turning was manually done every week during composting period. After 30 days of composting vermicomposting process was adopted.

Compost 3 (C_3) pit was filled by sundried sugarcane trash waste 2kg. 20g of *Trichoderma asperelloides* and 25 ml of microbial consortium was added. The same process was repeated till it reaches a height of above 1 meter. The moisture content of the pit was maintained for about 60-70% by springling of water in regular interval. To accelerate the composting process, manual turning was carried out every week during composting.

Sugarcane bagasse compost

The same procedure as presented above was repeated. Instead of sugarcane trash (C_1, C_2 and C_3), sugarcane bagasse was used in the following composting pits namely compost 4 (C_4) compost 5 (C_5) and compost 6 (C_6) respectively. Plate 8.

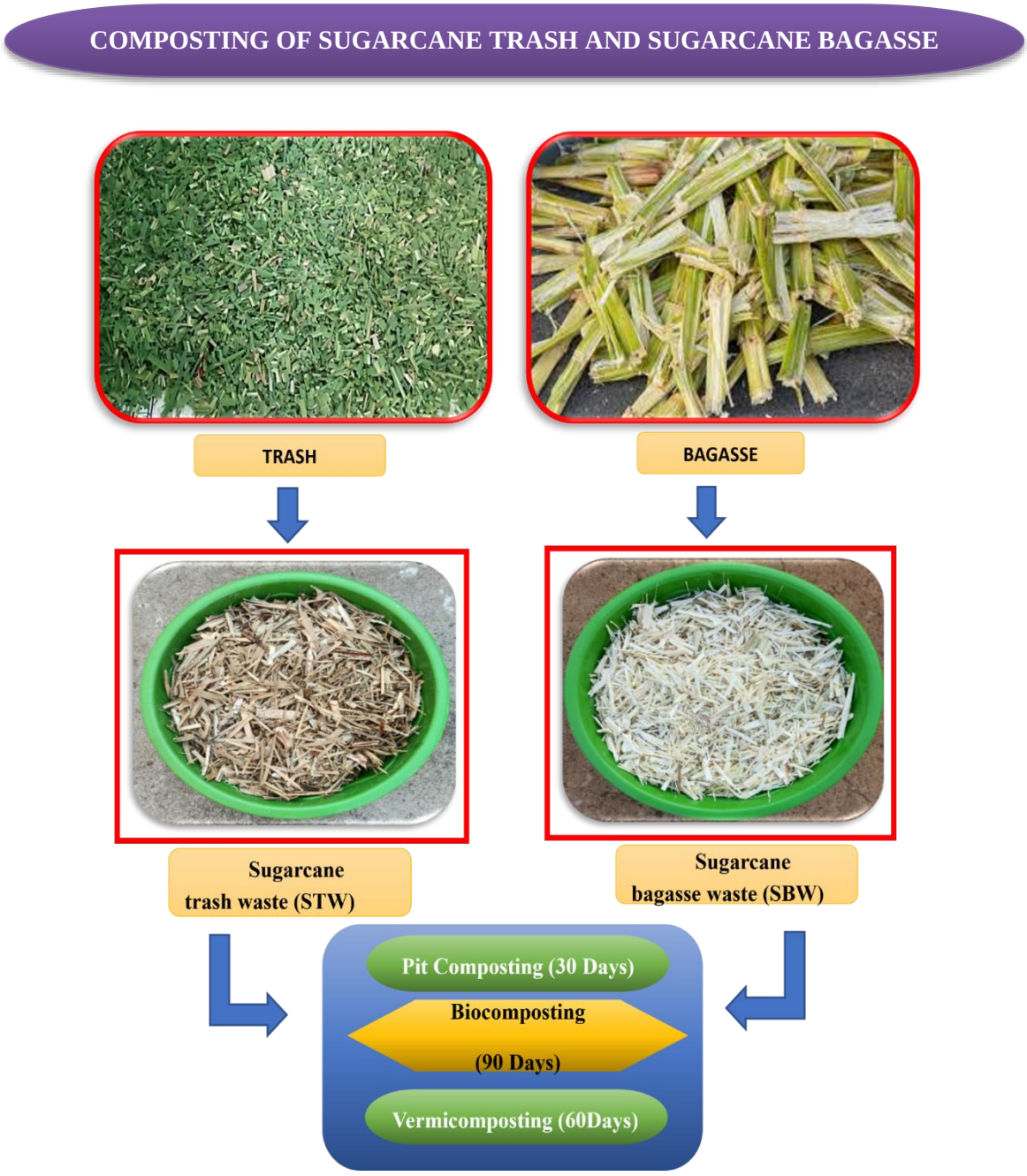
3.1.3 Experimental Preparation of Vermicomposting Trays by using *Eudrilus eugeniae* Kinberg

Plastic trays of 50×35×15cm were bought and holes were made at the bottom to drain the excess water in the experimental trays. Vermibeds were prepared by mixing the pre-decomposed sugarcane trash / sugarcane bagasse along with cow dung in ratio of 1:2 (1 kg of pre-decompost and 2 kg of cow dung) ratio on dry weight basis in the respected trays (C_1, C_2, C_4 and C_5). The substrates maintained 60- 80% of moisture content and kept for 24 h stabilization. Twenty-five healthy, clitellate individuals of exotic earthworms (*Eudrilus eugeniae* Kinberg) were inoculated into the respective designated experimental trays. The worms entered into the media immediately after the inoculation. Water was sprayed twice a day to maintain the moisture content. These experimental units were kept undisturbed in a shady place for 40 days. At the end of composting, the compost turned dark in color and the earthworms were separated by sieving. The collected vermicompost from each experimental tray were sieved with 3mm mesh size (Murali and Neelanarayanan, 2011).

3.2 EVALUATION OF COMPOST MATURITY

Physical and chemical analysis of composted agro-waste (sugarcane trash and sugarcane bagasse) was analyzed using standard methods. The following methods have been carried out for estimating the degree of maturity. The compost was analyzed for the following parameters.

PLATE 8



PHYSICAL PARAMETERS

pH and electrical conductivity of the composted raw and sugarcane trash and sugarcane bagasse were analyzed by using ELTOP digital meter and Deluxe conductivity meter 602.

CHEMICAL PARAMETERS

No	Chemical parameters	Reference	Appendix
1.	Lignin	Goering and Van soest, 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

3.2.1 Microbial Population during Composting

One gram of each sample was taken 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 10^6 with sterile distilled water as described by Kannan, (1996). One ml each of the dilutions of 10^6 (bacteria) and 10^4 (fungi) and 10^2 (actinobacteria) from each sample was transferred to sterile petri plates containing nutrient agar medium (bacteria), potato dextrose agar medium (fungi) and munaier's medium (actinobacteria) respectively for bacteria, fungi and actinobacteria in triplicates and incubated for one day and three days respectively. Microbial colony was counted during decomposition at regular interval of 30 days from 30, 60 and 90 days. The viable colony count was done with the help of colony counter.

3.2.2 FTIR Analysis

FT-IR spectra of raw and compost C_3 and C_5 (Best treatment) samples were dried at room temperature for one day to eliminate moisture content. A compost of finely ground 2 mg of homogenous sample was mixed with 400 mg of potassium bromide and compressed into pellets and recorded the transmittance response from 400 to 4000 cm^{-1} using FTIR (MIRACLE 10 Shimadzu).

PHASE II

3.3 FIELD EXPERIMENT

3.3.1 Experimental Site

Field experiment was conducted during August to November at Alanthurai (10.9536 N, 76.7885 E) Coimbatore, Tamil Nadu, India.

Field experiment was conducted with onion (*Allium cepa* L.), black nightshade (*Solanum nigrum* L.), tomato (*Solanum lycopersicum* L.) and brinjal (*Solanum melongena* L.) as the test crops to evaluate the effect of biocomposted sugarcane trash and sugarcane bagasse.

3.3.2 Soil Status

The soil type in this area is red soil. It has deep soil, good aggregate structure, abundant soil moisture, and good permeability.

3.3.3 Field Preparation

The field preparation was located in Alanthurai (10.9536 N, 76.7885 E) Coimbatore, Tamil Nadu, India. The field preparation was used a random block design, single row planted, row spacing was 35 cm, and planted spacing was 20 cm. The field treatments were designed as follows (Plate 9).

3.3.4 Collection of Seeds

Seeds of onion (*Allium cepa* L.), black nightshade (*Solanum nigrum* L.), tomato (*Solanum lycopersicum* L.) and brinjal (*Solanum melongena* L.) were collected from Tamil Nadu Agricultural University, Coimbatore.

3.3.5 Authentication of Test Crops

The plant samples,

Allium cepa L. (Identification No: BSI/SRC/5/23/2023-24/Tech/352) Annexure – 1, *Solanum nigrum* L. (Identification No: BSI/SRC/5/23/2022/Tech/570) Annexure – 2, *Solanum lycopersicum* L. (Identification No: BSI/SRC/5/23/2022/Tech/569) Annexure – 3, *Solanum melongena* L. (Identification No: BSI/SRC/5/23/2022/Tech/568) Annexure – 4, has been authenticated by Dr. M. U. Sharief, Scientist F & Head of office, Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore.

PLATE 9

FIELD PREPARATION

a **b** **c** **d**

a – Onion (*Allium cepa* L.) c – Tomato (*Solanum lycopersicum* L.)

b – Black Nightshade (*Solanum nigrum* L.) d – Brinjal (*Solanum melongena* L.)

3.3.6 Treatments Application and Cultivation

The compost about 5t/ha of prepared compost was incorporated into the soil to the respective plot according to the treatments and mixed thoroughly. About 10 viable seeds of onion (*Allium cepa* L.), black nightshade (*Solanum nigrum* L.), tomato (*Solanum lycopersicum* L.) and brinjal (*Solanum melongena* L.) were sown in each bed with three replications. After germination five to ten healthy plants were maintained per bed. Plant protection measures and other cultural practices were followed as per recommendation by Tamil Nadu Agricultural University, Coimbatore.

3.3.6 Treatment Details

TREATMENT DETAILS	
C	Control
T1	C_1 (Predecomposted Sugarcane trash + *Pleurotus florida* + *Eudrilus eugeniae* 5t/h)
T2	C_2 (Predecomposted Sugarcane trash + *Trichoderma asperelloides* + *Eudrilus eugeniae* 5t/h)
T3	C_3 (Predecomposted Sugarcane trash + *Trichoderma asperelloides* + Microbial consortium)
T4	C_4 (Predecomposted Sugarcane bagasse + *Pleurotus florida* + *Eudrilus eugeniae* 5t/h)
T5	C_5 (Predecomposted Sugarcane bagasse + *Trichoderma asperelloides* + *Eudrilus eugeniae* 5t/h)
T6	C_6 (Predecomposted Sugarcane bagasse + *Trichoderma asperelloides* + Microbial consortium)

3.4 BIOMETRIC PARAMETERS

3.4.1 Evaluation of Vegetative Characters of Test Crops

On the 30, 60 and 90 DAS onion (*Allium cepa* L.), black nightshade (*Solanum nigrum* L.), tomato (*Solanum lycopersicum* L.) and brinjal (*Solanum melongena* L.) plants were uprooted from the field and the following vegetative characters were noted.

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

3.5 Yield Parameters

On the 90th day, the plants were uprooted from the respective field and the following yield parameters were observed.

1. Number of fruits
2. Diameter of fruit
3. Length of the fruits
4. Single fruit weight
5. Fruit yield per plant
6. Fruit yield per plot

PHASE III

3.6 BIOCHEMICAL ANALYSIS

Biochemical parameters were analyzed in leaves and seeds of 30, 60 and 90 DAS.

No	Biochemical analysis	Reference	Appendix
1.	Protein	Lowry *et al*., 1951	Appendix – 8
2.	Carbohydrate	Hedge and Hofreiter, 1962	Appendix – 9
3.	Chlorophyll	Arnon, 1949	Appendix – 10

PHASE IV

3.7 EXPERIMENTAL PRE AND POST HARVEST SOIL SAMPLE ANALYSIS

No	Soil analysis	Method	Reference
1.	Available nitrogen	Alkaline permanganate	Subbiah and Asija, (1956) (Appendix - 11)
2.	Available phosphorus	Colorimetry	Jackson, (1973) (Appendix - 12)
3.	Available potassium	Flame photometry	Standford and English, (1949) (Appendix - 13)

3.8 SOIL MICROBIAL POPULATION

The soil samples were collected in separate sterilized samples bottles. Each sample bottle was labelled properly with land use types, location, collection date and taken to the laboratory for microbial analysis. The number of soil microorganisms was determined using the dilution spread plate technique. Nutrients agar (NA), Potato Dextrose agar (PDA) and Munaier's medium (MM) were the culture media of choice used for bacteria, fungi and actinobacteria. One milliliter aliquot of sample was pipetted into sterile test tube and serially diluted in another six set of test tubes each containing 9ml of sterile distilled water to dilution ratio 10^{-6}., 0.1 ml portion of the diluents from the fourth (10^{-4}), fifth (10^{-5}) and fourth (10^{-4}) dilution factors were pipette separately aseptically into different sterile petri dishes and 20 ml of the cool (45^0C) sterile molten agar media was added under aseptic condition, swirled gently for even distribution of the inoculate, allowed to set and incubated at 30-37^{0}C for 24 hours (for bacterial), at 25-27^{0}C for 72 hours (for fungi), and at 30-37^{0}C for 144 hours (for actinobacteria). At the end of incubation (24 hours) microbial colony were counted and recorded appropriately for bacteria while after (72 hours) microbial colony were counted appropriately for fungi and (144 hours) microbial colony were counted and recorded for actinobacteria respectively.

3.9 ANTIOXIDANT ACTIVITY

No	Antioxidant activity	Reference	Appendix
1.	DPPH	Mensor *et al*., 2001	Appendix – 14
2.	Hydrogen peroxide scavenging activity	Ruch *et al*., 1989	Appendix - 15
3.	Reducing power assay	Green *et al*., 1982	Appendix – 16
4.	Nitric oxide inhibition assay	Oyaizu, 1986	Appendix – 17

3.10 ANTIBACTERIAL ACTIVITY (Bauer *et al*., 1966) Appendix-18

Statistical Analysis

The data obtained on biometrical observations (root length, shoot length, number of leaves, number of flowers / plant, number of branches per / plant, fresh weight and dry weight), biochemical analysis (protein, carbohydrates and chlorophyll on 30, 60 and

90 DAS) and yield parameters (number of fruits/ plant, diameter of fruit, length of fruits, single fruit weight, fruit yield per plant and fruit yield per plot plant) on 90th day, antioxidant and antibacterial activity in seed extract of the best treatment of the test crops were subjected to the statistical analysis (one way and two way ANOVA) and based on the results inference were drawn.