Methodology

3 METHODOLOGY

The present society with its high population density, heavy industrialization and intensive methods of agriculture produces ever increasing quantities of solid wastes (ICRA, 2006). About 40–60% of these solid wastes in India are organic in nature. Open dumping of such wastes not only facilitates the breeding of disease vectors like flies, mosquitoes, cockroaches, rats and other pests, but at the same time also creates environmental pollution.

Disposal of waste is becoming one of the major areas of concern for developing countries like India. The application of undecomposed wastes or non-stabilized compost to land may lead to immobilization of plant nutrients and phytotoxicity (Butler *et al.,* 2001). One of the methods to overcome this problem is recycling of the wastes which are an efficient and environment-friendly technology to get value-added products.

Sago, the edible starch globules processed from the tubers of tapioca is the staple diet of the middle income groups in India. Nearly 60% of the tapioca in India is used industrially for the production of sago, starch and dry chips (Srinivas, 2007). In the southern regions of India alone, there are about 1000 sago and starch processing factories producing 15–30 tonnes of sago per unit per day (Saravanane *et al.,* 2001).

Sago processing is generally perceived as polluting the environment. Processing of tapioca requires 20,000 to 30,000 litres of water per ton. Besides, it produces equal quantities of a highly organic, foul smelling and acidic waste water alongwith the sago sludge which is called '*thippi*' in Tamil (Banu *et al.,* 2006a). Hence, it is necessary to manage these huge quantities of biodegradable solid waste in an ecofriendly manner.

Composting is one of the most promising ways of organic waste management to recycle the wastes as the process reduces the volume and stabilizes the waste. Although thermocomposting has been adopted as a basic tool for onsite waste decomposition, there are some disadvantages of this traditional method, such as, long duration of the process, frequent requirement of aeration, loss of nutrients (like gassing off of nitrogen) and heterogeneous end products (Nair *et al.,* 2006).

Vermicomposting is a suitable technology employing earthworms for decomposition of different types of organic wastes (domestic as well as industrial) into value added materials (Ravikumar *et al.,* 2008). The end product of vermicomposting which is called vermicompost is rich in essential macro and micronutrients alongwith microorganisms in a very simple form that can be easily taken up by plants (Parthasarathi *et al.,* 2007).

With this background information, the present study entitled "Biocomposting of tapioca solid waste with selected earthworms and microbes" was taken up with the objective of composting tapioca solid waste with earthworms and microorganisms and studying the physicochemical and biochemical parameters in the composts obtained and also analyzing the effect of the composts on the growth of selected plants.

The experimental design of the study was as follows:

3.1 PHASE I : COMPOSTING OF TAPIOCA SOLID WASTE

3.1.1 COLLECTION OF SUBSTRATES

3.1.1.1 Tapioca Solid Waste

3.1.1.2 Cow dung

3.1.2 COLLECTION OF EARTHWORMS

3.1.3 COLLECTION OF MICROBIAL CULTURE

3.1.4 COMPOSTING

3.1.4.1 Natural composting

3.1.4.2 Microbial composting

3.1.4.3 Vermicomposting

3.1.4.4 Microbial Vermicomposting

(Composting using earthworms and microorganisms)

3.1.5 HARVEST OF COMPOSTS

3.1.6 DETERMINATION OF PHYSICOCHEMICAL AND BIOCHEMICAL PARAMETERS IN COMPOSTS.

3.1.6.1 pH

3.1.6.2 Electrical conductivity

3.1.6.3 Organic matter and organic carbon

3.1.6.4 Total nitrogen

3.1.6.5 Carbon nitrogen ratio

3.1.6.6 Total phosphorus

3.1.6.7 Total potassium

3.1.6.8 Available nitrogen

3.1.6.9 Available phosphorus

3.1.6.10 Available potassium

3.1.6.11 Sodium

3.1.6.12 Calcium

3.1.6.13 Magnesium

3.1.6.14 Sulphur

3.1.6.15 Trace elements

3.2 PHASE II : ANALYSIS OF THE COMPOSTS

3.2.1 SELECTION OF SUBSTRATE

3.2.2 COMPOSTING OF 3:1 RATIO SUBSTRATE

3.2.3 HARVEST OF COMPOSTS

3.2.4 ASSAY OF SELECTED ENZYMES IN THE COMPOSTS

3.1.4.1 Cellulase

3.1.4.2 β- glucosidase

3.1.4.3 Protease

3.1.4.4 Urease

3.1.4.5 Dehydrogenase

3.1.4.6 Phosphatase

3.2.5 ASSESMENT OF COMPOST MATURITY

3.2.5.1 Humic acid

3.2.5.2 Carbondioxide evolution

3.2.5.3 Earthworm biomass

3.2.5.4 Earthworm number and cocoon number

3.2.6 COMPOST YIELD

3.3 PHASE III : ASSESMENT OF THE EFFECT OF COMPOST ON PLANT GROWTH

3.3.1 SELECTION OF COMPOST FOR PLANT STUDY

3.3.2 SELECTION OF THE PLANTS

3.3.3 PREPARATION OF THE POT MIXTURES

3.3.4 DETERMINATION OF BIOMETRIC PARAMETERS

3.1.4.1 Germination index

3.1.4.2 Root and shoot lengths

3.1.4.3 Root fresh and dry weights

3.1.4.4 Shoot fresh and dry weights

3.1.4.5 Nodule number

3.1.4.6 Seed number

3.3.5 ESTIMATION OF BIOCHEMICAL PARAMETERS

3.3.5.1 Soluble sugar

3.3.5.2 Protein

3.3.5.3 Amino acids

3.4 STATISTICAL ANALYSIS

3.1 PHASE I : COMPOSTING OF TAPIOCA SOLID WASTE

3.1.1 COLLECTION OF SUBSTRATES

3.1.1.1 Tapioca Solid Waste

Tapioca Solid Waste (TSW) is a fibrous residue termed as *'thippi'* in Tamil. The *'thippi'* is generated at the rate of 15-20% per ton of the tapioca tubers processed. The accumulation of *'thippi'* near sago industrial areas leads to foul odour due to microbial action causing the growth of undesirable microflora (Christy and Ramalingam, 2005). The Tapioca Solid Waste (TSW) was collected from Logesh Sago Factory, Harur - Pudupatti, Dharmapuri District, Tamil Nadu. It was dried and powdered to reduce the particle size. Plate 1 shows the undecomposed tapioca solid waste.

PLATE 1

UNDECOMPOSED TAPIOCA SOLID WASTE

3.1.1.2 Cow dung

The bulking materials (co substrates for vermicomposting) play an important role in the process of vermicomposting of organic wastes. The type and proportion of a bulking material as co substrate not only influences the mineralization rate but at the same time also alters the earthworm biomass and production rate. Cattle dung, which is a bulking material, contains a variety of microorganisms which may accelerate the mineralization process through enzyme synthesis. Several results clearly suggest that cow dung may be an efficient bulking agent for rapid decomposition of waste through vermicomposting (Suthar and Singh 2008). Cow dung was thus selected for the

present study. It was collected from a cattle farm house in Thiruchengode, Namakkal District, Tamil Nadu, dried and powdered. Plate 2 shows the undecomposed cow dung.

PLATE 2

UNDECOMPOSED COW DUNG

3.1.2 COLLECTION OF EARTHWORMS

Three species of earthworms (*Eisenia fetida*, *Perionyx excavatus* and *Lampito mauritii*) have been consistently used for commercial composting due to their high tolerance of environmental variations.

These species are highly adaptable and can tolerate varying degrees of moisture. *Eisenia fetida is the* most commercially used earthworm for vermicomposting, *Perionyx excavatus* is reported to produce excellent changes in the organic waste (Suthar, 2007a) and *Lampito mauritii* effectively creates a drilosphere apart from helping in compost production (Parthasarathi and Ranganathan, 2000). Therefore, the above three types of earthworms (*Eisenia fetida*, *Perionyx excavatus*, and *Lampito mauritii*) were chosen for the experiment. These earthworms were procured from a vermicompost producing centre maintained by the Self Help group at Kokkarayanpettai, Thiruchengode, Namakkal District, Tamil Nadu, India.

3.1.3 COLLECTION OF MICROBIAL CULTURE

To exploit the organic resources, microorganisms mutually associate with macroorganisms (earthworms). Microorganisms are able to perform any chemical transformation during the decomposition of organic materials but their activity is highly dependent on macroorganisms. Free soil microorganisms find suitable conditions for their activity in the anterior part of the earthworm gut as well in the worm casts. Many research workers have reported that there are greater numbers of microorganisms in earthworm casts than in surrounding soil (Prakash *et al.,* 2008).

Interaction between earthworms and microorganisms is essential for the degradation of organic matter and release of nutrients (Aira *et al.,* 2002). Fresh cultures of microbial broth containing *Tricoderma viridae* and *Bacillus polymyxa* were prepared from the stock culture. It was purchased from the Department of Microbiology, Sengunthar Arts and Science College, Tiruchengode, Namakkal District, Tamil Nadu, India.

3.1.4 COMPOSTING

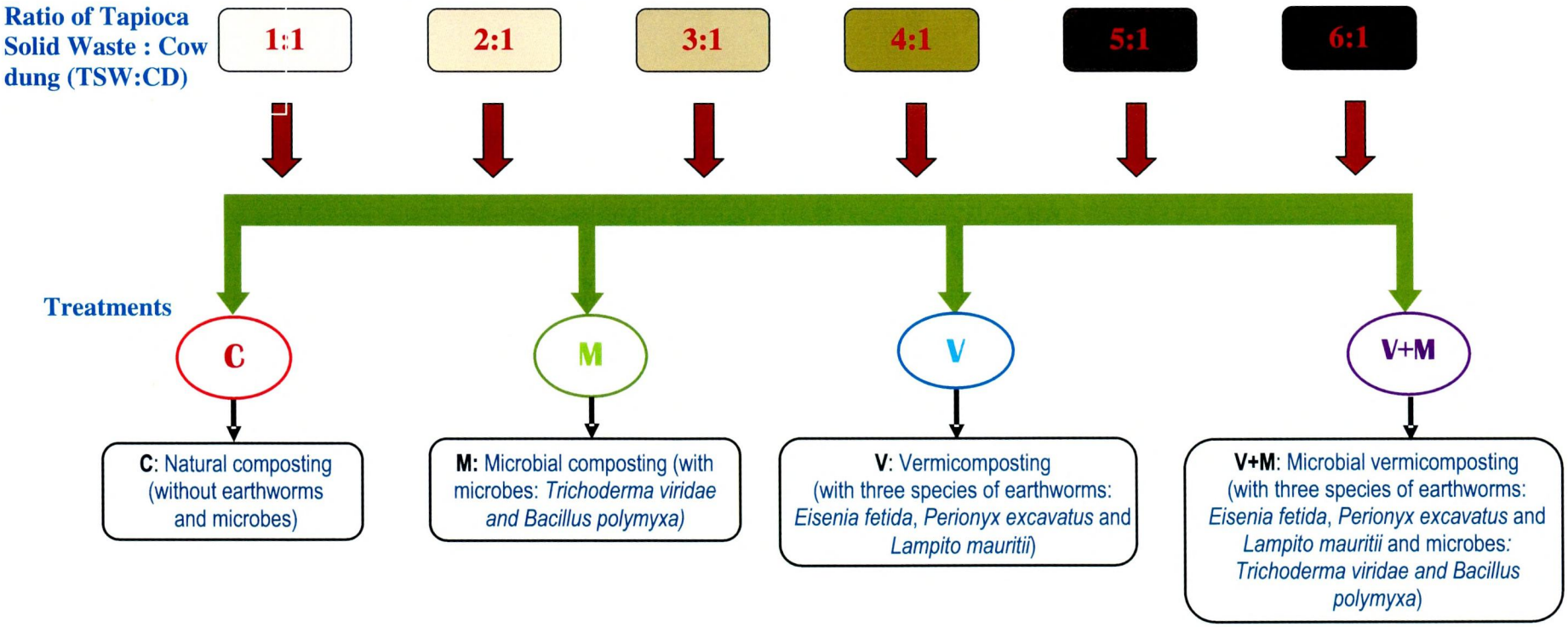
Composting of the tapioca solid waste was carried out as follows.

Cement tanks of dimension 0.5×0.3×1.0 m size with small perforations at the bottom were taken for the composting. 5 kg of the substrate mixture containing varying proportions of tapioca solid waste and constant amounts of cow dung (1:1, 2:1, 3:1, 4:1 5:1 and 6:1) were taken in each tank. For the better survival of earthworms, the substrate mixtures were precomposted for a period of 20 days.

At the end of the 20th day, four treatments were given for each ratio of tapioca solid waste and cow dung as given in Figure 2.

FIGURE 2

COMPOSTING OF TAPIOCA SOLID WASTE



3.1.4.1 Natural composting

Natural composting **(C)** was allowed to take place for a period of 75 days. During this type of composting, decomposition of the organic waste matter accurs naturally due to the presence of residual microorganisms in the waste.

3.1.4.2 Microbial composting

For microbial composting **(M),** a mixture of two types of microorganisms, namely, *Tricoderma viridae* and *Bacillus polymyxa* (50ml each) were mixed with the substrate and kept for composting for 75 days (Manna *et al.,* 2003).

3.1.4.3 Vermicomposting

Vermicomposting **(V)** was carried out with a mixture of three types of earthworms, namely, *Eisenia fetida*, *Perionyx excavatus*, and *Lampito mauritii.* These earthworms were mixed with the substrate (10 numbers per kg) and left aside for 75 days for composting (Christy and Ramalingam, 2005).

3.1.4.4 Microbial Vermicomposting

Composting using both earthworms and microorganisms (M+V) hastens the decomposition process. Three types of earthworms - *Eisenia fetida*, *Perionyx excavatus*, and *Lampito mauritii* (10 numbers per kg) + two types of microorganisms - *Tricoderma viridae* and *Bacillus polymyxa* (50ml culture per kg) were mixed with the substrates and allowed to compost for a period of 75 day (Pramanik *et al.,* 2007).

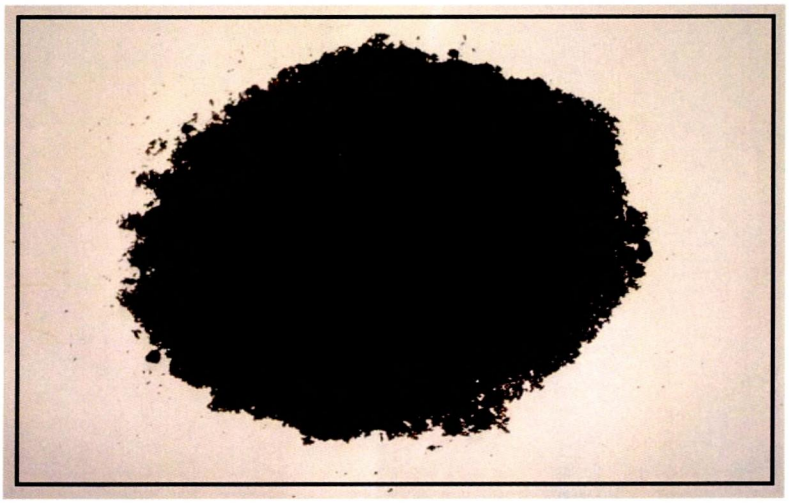
After the above treatments, all the tanks were covered with nylon net and kept in the shade away from sunlight at room temperature. The wastes were carefully turned over twice a week. Water was sprinkled once in three days on the wastes to maintain humidity.

3.1.5 HARVEST OF COMPOSTS

Moistening of the composts was suspended for two days before harvest so as to allow the composts to dry. On the 75th day, the compost from each tank was harvested. The vermicomposts were placed in the form of a cone on the ground in sunlight for one hour in order to allow the earthworms to settle down at the bottom. The earthworms were then removed and the composts collected. They were then dried, sieved and used for analysis. Plate 3 shows the compost formed from the 3:1 ratio tapioca solid waste - cow dung mixture.

PLATE 3

MICROBIAL VERMICOMPOST FORMED FROM 3:1 RATIO TAPIOCA SOLID WASTE - COW DUNG MIXTURE



3.1.6 DETERMINATION OF PHYSICOCHEMICAL AND BIOCHEMICAL PARAMETERS IN COMPOSTS

3.1.6.1 pH

Different substrates may show different behavior during pH shift and results in the production of various intermediates (Gupta and Garg, 2009). The pH of the various composts and their raw substrates was determined by using a pH meter (ISI Bulletin, 1982). The procedure for the same is given in Appendix 1.

3.1.6.2 Electrical conductivity

Electrical conductivity reflects the degree of salinity of the compost indicating its possible phytotoxic effects on the growth of plants (Kalamdhad *et al.*,2009). The determination of electrical conductivity was done using a conductometer (ISI Bulletin, 1982). The procedure for the same is explained in Appendix 2.

3.1.6.3 Organic matter and organic Carbon

In composting, organic matter transfer occurs in terms of carbondioxide evolution as well as water loss by evaporation leading to changes in the organic matter and organic carbon contents of the composts (Romerio *et al.,* 2007). Therefore, determination of organic matter and organic carbon contents in the composts is very essential. These parameters were estimated by Walkey Modified Procedure of Potassium Dichromate Oxidation Method (Nelson and Sommers, 1982; Walkley and Black, 1934) as detailed in Appendix 3.

3.1.6.4 Total nitrogen

During composting, earthworms and microorganisms enhance nitrogen mineralization in the substrate so that the mineral nitrogen is retained in the nitrate form (Atiyeh *et al.,* 2000). The determination of total nitrogen is therefore important. It was determined by Microkjeldahl method (Tandon, 1993). The protocol is given in Appendix 4.

3.1.6.5 Carbon nitrogen ratio

A change in C/N ratio reflects the extent of organic matter decomposition and stabilization achieved during composting (Nair *et al.,* 2006). This was calculated from the carbon and nitrogen values of the composts.

3.1.6.6 Total phosphorus

The release of phosphorus in the available form is performed partly by earthworm gut phosphatases and further release might be attributed to the

phosphate solubilizing microorganisms present in worm casts (Zularisam *et al.,* 2010). The total phosphorus levels in the composts were determined by colorimetric method as given by Tandon (1993). The procedure for the same is depicted in Appendix 5.

3.1.6.7 Total potassium

In composting, the presence of a large number of microflora may play an important role in changing the potassium content of the compost (Kaviraj and Sharma, 2003). This element was estimated by Flame Photometry (Tandon, 1993) and the procedure is given in Appendix 6.

3.1.6.8 Available nitrogen

An important feature of vermicomposting is that during the processing of the various organic wastes by earthworms, many of the nutrients are converted into available forms that are more readily taken up by plants such as nitrate or ammonium nitrate (Suthar and Singh, 2008). The available nitrogen in the composts and vermicomposts were determined by titrimetric method as described by Subbiah and Asija (1956).The protocol in detailed in Appendix 7.

3.1.6.9 Available phosphorus

The total available phosphate changes during the vermicomposting process (Sangwan *et al.,* 2008). The determination of available phosphorus in the compost was done by the Method of Olsen *et al.* (1954). The protocol for this is depicted in Appendix 8.

3.1.6.10 Available potassium

Changes in the available potassium contents also occur during vermicomposting. Its level in the composts and vermicomposts were determined by Flame Photometric Method as described by Hanway and Heidel (1952). The procedure for this is recorded in Appendix 9.

3.1.6.11 Sodium

The sodium levels in the samples were determined by Flame Photometric Method as explained by Tandon (1993). The method for the same is explained in Appendix 6.

3.1.6.12 Calcium

The enzymes in earthworms and microorganisms are the responsible factors for changing the levels of the calcium during the composting process (Suthar, 2008). The calcium levels were estimated by Flame Photometric Method (Tandon, 1993) and the protocol is explained in Appendix 6.

3.1.6.13 Magnesium

Composting alters the magnesium contents of the substrates (Ramalingam and Thilagar, 2003). This was determined by Atomic Absorption Spectrophotometric method, (Krishna and Ranjan, 1991). The protocol for the same is recorded in Appendix 11.

3.1.6.14 Sulphur

The sulphur levels in the composts and substrates were determined by Gravimetric method edited by Raghuramulu *et al.* (1983). The procedure for this is given in Appendix 10.

3.1.6.15 Trace elements

The trace (micronutrient) elements such as iron, zinc, copper, molybdenum and boron are essential for adequate growth and reproduction of plants. Despite their low concentrations in the plant tissues, micronutrients are of equal importance as macronutrients which are essential for the growth and development of plants (Kirkby and Romheld, 2004). During vermicomposting, the trace element levels significantly change in the final product of the

composting process (Adi and Noor, 2009). The levels of these elements (magnesium, iron, zinc, copper, molybdenum and boron) were estimated by Atomic Absorption Spectrophotometric Method; (Krishna and Ranjan, 1991) Appendix 11 explains the technique for the estimation.

3.2 PHASE II : ANALYSIS OF THE COMPOSTS

3.2.1 SELECTION OF SUBSTRATE

The physicochemical analysis of the composts obtained in Phase I revealed that the 3:1 ratio of tapioca solid waste – cow dung composts showed the best results as regards most of the treatments. Hence, this ratio was selected for further study in this phase.

3.2.2 COMPOSTING OF 3:1 RATIO SUBSTRATE

The composting of the 3:1 ratio tapioca solid waste – cow dung mixtures was carried out as follows.

Cement tanks of dimension 0.5×0.3×1.0m size with small perforations at the bottom were taken for the composting. 5 kg of the substrate mixture was taken in each tank. For the better survival of earthworms, the substrate mixtures were precomposed for a period of 20 days.

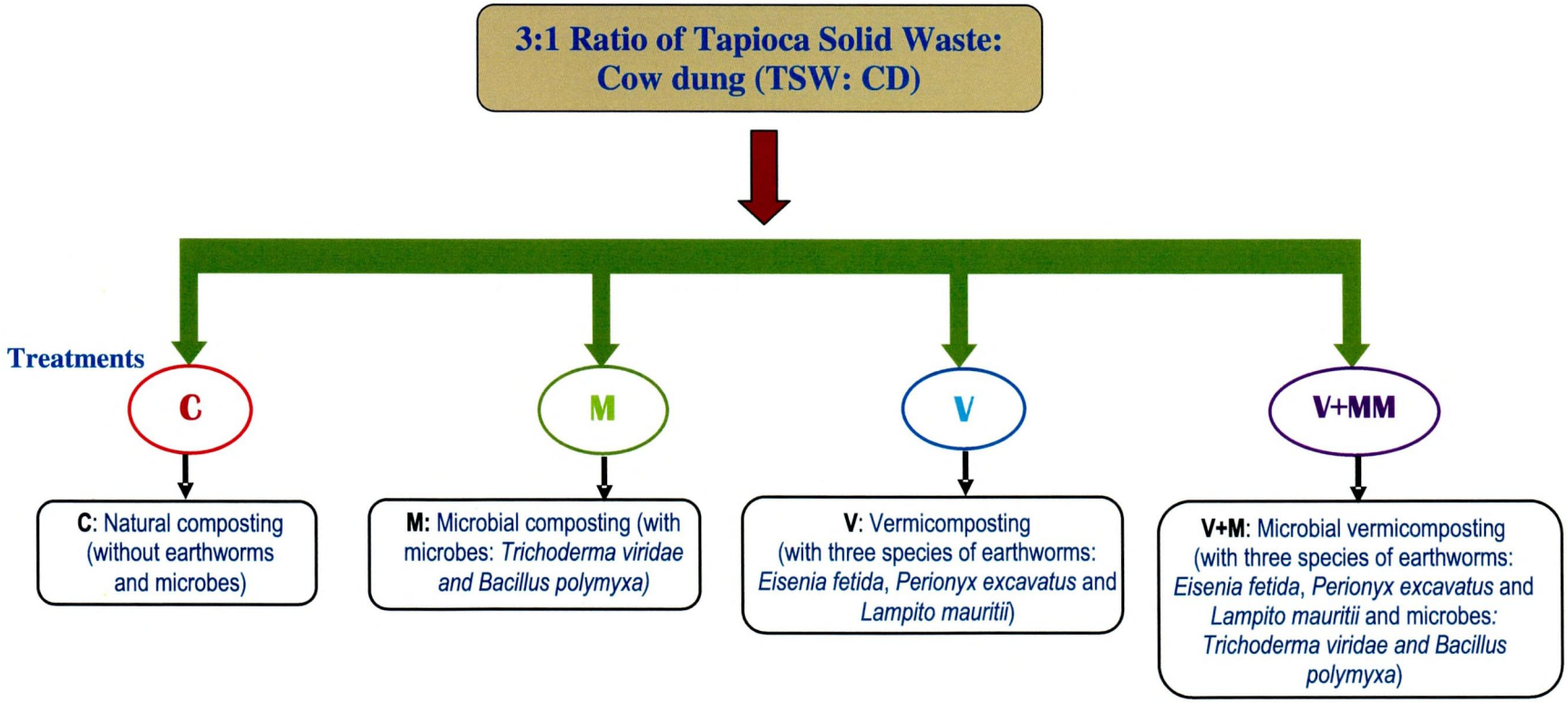
At the end of the 20^{th} day, four treatments were given as shown in Figure 3 This was carried out as for Phase I (steps 3.1.4.1 to 3.1.4.4).

3.2.3 HARVEST OF COMPOSTS

The composts were collected at specific intervals - on the 25^{th}, 50^{th} and 75^{th} days of composting. The earthworms were removed from the vermicomposts. All the composts were then dried, sieved and used for the analysis.

FIGURE 3

COMPOSTING OF 3 : 1 RATIO TAPIOCA SOLID WASTE - COW DUNG MIXTURE



3.2.4 ASSAY OF SELECTED ENZYMES IN THE COMPOSTS

Enzymatic activity is due to enzymes in living or dead cells, cell debris, free enzymes and/or enzymes adsorbed by clay or immobilized in humus complexes (Nannipieri *et al.*, 2002). Vermicompost ageing is correlated with enzyme activities at various stages of composting, because this parameter can control the quality of the resulting compost. A study of the enzyme profile during various stages of composting reveals the extent of nutrient transformation and maturation of the substrate.

3.2.4.1 Cellulase

During composting, cellulolysis occurs as a result of the combined action of fungi and bacteria with different requirements. Earthworms influence decomposition indirectly by affecting the microbial population, structure and dynamics and also directly, because, the guts of some species possess cellulolytic activity (Aira *et al.,* 2005). The cellulase in the composts was determined. The cellulase action was estimated by the Method given by Schinner and Von Mersi, (1990) except that the reducing sugar released in the filtrate was analyzed by the method of Denision and Koehn, (1977) as explained by Sadasivam and Manickam (2008). The procedure for this is given in Appendix 12.

3.2.4.2 β-glucosidase

β-glucosidase catalysis the hydrolysis of cellobiose and plays a major role in the decomposition of organic compounds during composting (Cayuela *et al.,* 2008). Appendix 13 gives the protocol for assay the β-glucosidase activity as explained by Eivazi and Tabatabai, (1988).

3.2.4.3 Protease

Protease action is important in composting, because, it catalyses the depolymerization of nitrogen - containing compounds into dissolved organic

nitrogen which is a critical step in the nitrogen cycle (Schimel and Bennet, 2004). Protease activity in the composts was assayed by the method given by Ladd and Butler (1972) and Mahadevan and Sridhar (1996). Appendix 14 gives the method for the assay.

3.2.4.4 Urease

Alteration in urease activity in the compost might be due to increase in extracellular activity during the controlled biodegradation of the wastes. This may be due to the continous accumulation of cell released (extracellular) enzymes in humic matter which become stabilized and resistant to physical and microbial degradation (Benitez *et al.*, 2000). The urease activity was estimated by the method given by Pancholy and Rice, (1973) except that the ammonia liberated in the reaction mixture was assessed by nesslerisation as described by Jackson (1973). The procedure is explained in Appendix 15.

3.2.4.5 Dehydrogenase

Dehydrogenase activity measures the intracellular catalysis and is more likely to be correlated with the activity of all microorganisms in the composting process (Atiyeh *et al.,* 2001). It was estimated by the method given by Casida *et al.* (1964), as recorded in Appendix 16.

3.2.4.6 Phosphatase

The activity of phosphatases was analyzed due to their involvement in the phosphorus cycle. The microorganisms entering into the earthworm gut consume nitrogenous compounds of the mucus which largely increase phosphatase activity enabling them to contribute enzymes during the digestive process of the earthworms (Zhang *et al.,* 2000).

The activities of acid and alkaline phosphatases were determined by the method given by Tabatabai and Bremner (1969). The procedure is presented in Appendix 17.

3.2.5 ASSESMENT OF COMPOST MATURITY

Maturation of the product is a critical step during composting. This begins once earthworms leave the substrate. It is a microbe-driven process. The microbiological properties of compost, such as, microbial biomass content and associated activities regulate nutrient dynamics during maturation, leading to immobilization or release of nutrients (Schimel and Bennet, 2004). The effects of compost ageing, especially patterns of carbondioxide evolution, humic acid level, earthworm biomass, cocoon formation and yield of the composts were investigated because these parameters control the quality of the resulting composts (Bajsa *et al.,* 2004).

3.2.5.1 Humic acid

Earthworms fragment organic substrates, stimulate microbial activity and increase rates of mineralization rapidly converting the wastes into humus-like substances (Atiyeh *et al.,* 2000). The decomposition of organic matter is characterized by the conversion of part of the organic material into humic substances (Castaldi *et al.,* 2005). Humic acid was determined using Alkali Acid Fractionation Method (Valdrighi *et al.,* 1996). The protocol for the same is detailed in Appendix 18.

3.2.5.2 Carbondioxide evolution

Carbondioxide evolution is the most direct technique to assess compost stability, because, it measures the carbon derived directly from the compost being tested. Carbondioxide evolution in the composts was estimated by the method given by Anderson, (1982); Dubey and Maheshwari, (2002). The procedure is presented in Appendix 19.

3.2.5.3 Earthworm biomass

After counting the number of earthworms and their cocoons as mentioned above, the earthworms were subsequently weighed on a weighing scale before returning them back into the composting tank.

3.2.5.4 Earthworm number and cocoon number

On the 25th, 50th and 75th days of composting, the earthworms from the vermicomposting and microbial vermicomposting tanks were separated by hand sorting, after which they were washed in tap water to remove the adhering material from their body and counted. The numbers of cocoons were also counted. The earthworms were then introduced back into the respective composting tanks.

3.2.6 COMPOST YIELD

The composts harvested on the 75th day were weighed to get the yield.

3.3 PHASE III : ASSESMENT OF THE EFFECT OF COMPOST ON PLANT GROWTH

Several studies have showed that application of vermicompost promotes the growth of plants (Canellas *et al.,* 2002). Improvement in plant growth and increase in yield may be due to increase in microbial biomass in soils receiving vermicompost which increases nutrient mineralization and converts unavailable forms of nutrients into available forms. Moreover, the increase in microbial biomass also suppresses plant pathogens (Arancon *et al.,* 2002) and plant diseases (Chaoui *et al.,* 2002).

3.3.1 SELECTION OF COMPOST FOR PLANT STUDY

The results of Phase II clearly reveal that the quality of the 3:1 ratio microbial vermicompost (M+V) was better than those of the other composts. Hence, it was decided to select this compost for the plant studies.

3.3.2 SELECTION OF THE PLANTS

Two different types of plants - green leafy vegetable (Fenugreek - *Trigonella foenum gracum*) and leguminous plant (Black gram - *Vigna mungo*) were selected to find out the effect of the microbial vermicompost (M+V) supplemented soil on their growth characteristics.

3.3.3 PREPARATION OF THE POT MIXTURES

Garden soil was supplemented with 20, 40, 60, and 80 percent (w/w) of the 3:1 ratio microbial vermicompost and mixed well (Arancon *et al.,* 2004). 2 kg each of the supplemented soil was taken in different pots. 5 replicate pots were taken for each type of vermicompost - supplemented soil. 20 seeds of fenugreek were sown into one set of pots and 10 seeds of black gram into another set of pots and allowed to germinate and grow. In the case of fenugreek, all the germinated plants were allowed to grow for a period of 10 days. As regards black gram, the germinated plants which exhibited the maximum growth by the 10^{th} day were allowed to continue growing for a period of 90 days.

Figure 4 depicts the experimental design of Phase III

3.3.4 DETERMINATION OF BIOMETRIC PARAMETERS

3.3.4.1 Germination index

The germination index was calculated by the method described by Mathur *et al.,* (1993). The procedure is presented in Appendix 20.

3.1.4.2 Root and Shoot lengths

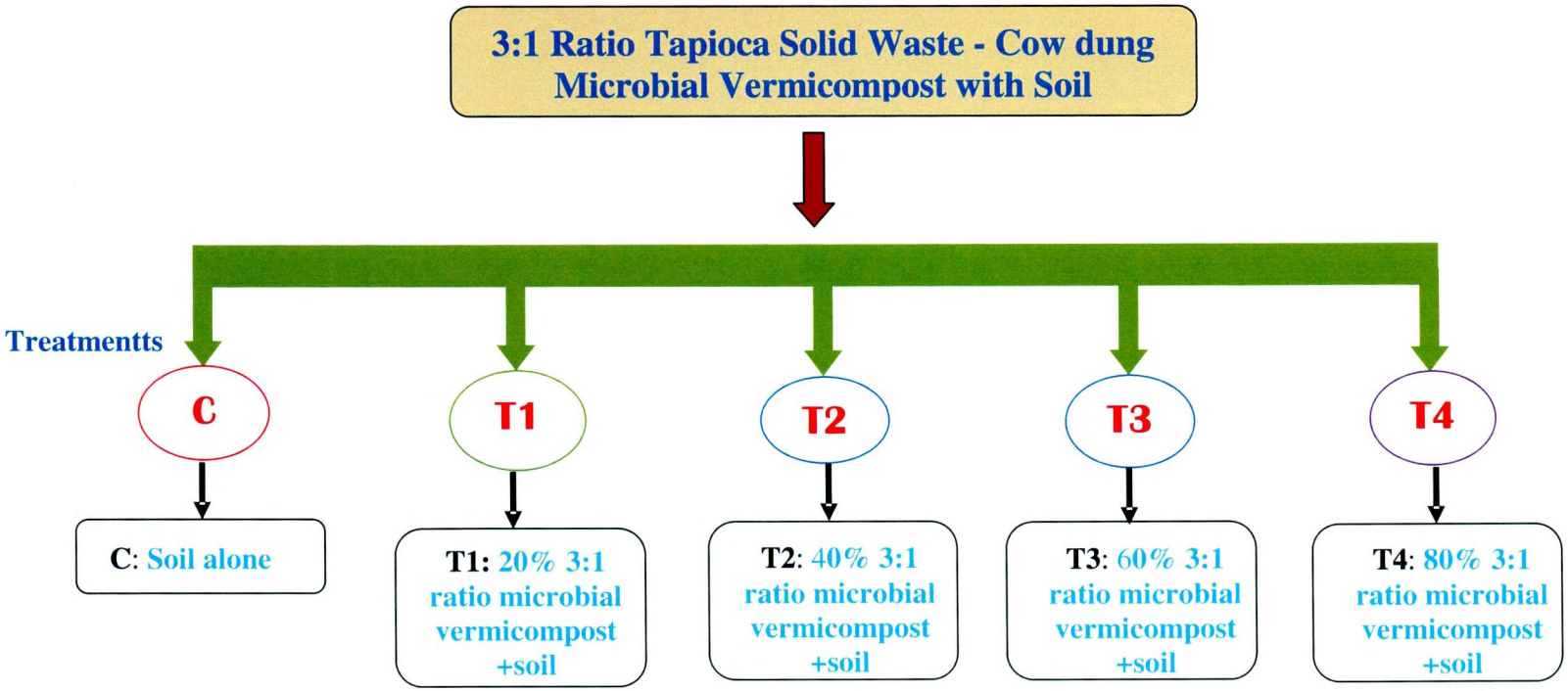
The root and shoot lengths of each plant were measured on the 90^{th} day for black gram and 10^{th} day for fenugreek. The procedure for the same is given in Appendix 21.

3.1.4.3 Root fresh and dry weights

The fresh and dry weights of the roots were taken. The procedure (Ali *et al.,* 2007) for this is explained in Appendix 22.

FIGURE 4

APPLICATION OF DIFFERENT PROPORTIONS OF 3:1 RATIO TAPIOCA SOLID WASTE – COW DUNG MICROBIAL VERMICOMPOST WITH SOIL FOR PLANT GROWTH



3.1.4.4 Shoot fresh and dry weights

The fresh and dry weights of the shoots were taken. The procedure (Ali *et al.*,2007) for this is explained in Appendix 22.

3.1.4.5 Nodule number

The number of nodules formed on the black gram plants were counted on the 60^{th} day of growth.

3.1.4.6 Seed number

The number of seeds present in the black gram plant were noted upto the 90^{th} day of growth.

3.3.5 ESTIMATION OF BIOCHEMICAL PARAMETERS

3.3.5.1 Soluble sugar

The soluble sugar content of the *Fenugreek* plants was determined by Anthrone method (Mahadevan and Sridhar, 1996). Appendix 23 gives the procedure for the estimation.

3.3.5.2 Protein

The protein content of the plant (Fenugreek) was determined by Lowry's Method (Sadasivam and Manickam, 2008) and the procedure is given in Appendix 24.

3.3.5.3 Amino acids

The amino acid contents of the plants (Fenugreek) were estimated by Ninhydrin method (Sadasivam and Manickam, 2008). The technique is explained in Appendix 25.

3.4 STATISTICAL ANALYSIS

The data obtained from the above were analyzed by t-test, two way ANOVA and Duncan's Multiple Range Test (DMRT) to find out the statistically significant differences in the parameters studied among the different treatment groups.

The observations made at different stages of the study and the results obtained thereof are presented and discussed in the following chapter.