CHAPTER III

3. MATERIALS AND METHODS

3.1 TYPES OF ORGANIC WASTES USED

Agricultural residues (Plate 1) such as paddy straw (Oryza sativa), pods of soyabeans (Glycine max), blackgram (Phaseolus mungo), greengram (Phaseolus aureus) and maize stalk (Zea mays) were collected from the roadside and agriculture field during harvesting time in and around Courtallam and Tenkasi in Tirunelveli District, Tamil Nadu, India (Map 3:1:1). The Industrial waste viz., waste cotton (Gossypium species; Plate 1) was collected from the Cotton Mills, Rajapalayam, Virudhunagar District, Tamil Nadu, India (Map 3:1:1). The collected Agricultural residues were utilized for mushroom cultivation, Pleurotus sajor-caju.

3.2. SYSTEMATIC POSITION (Alexopoulose and Mims, 1993)

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Myceteae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Amastigomycota</td>
</tr>
<tr>
<td>Subdivision</td>
<td>Basidiomycotina</td>
</tr>
<tr>
<td>Class</td>
<td>Basidiomycetes</td>
</tr>
<tr>
<td>Subclass</td>
<td>Holobasidiomycetidae</td>
</tr>
<tr>
<td>Order</td>
<td>Agaricales</td>
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3.3. CULTIVATION OF MUSHROOM, *Pleurotus sajorcaju*

*Pleurotus sajorcaju* also known as oyster mushroom or Dhingri is a well-known edible saprophytic basidiomycetous fungus. It was found to grow naturally on succulent tissues of *Euphorbia roylenasbioss* in the foothills of the Himalayas. Pure culture of *Pleurotus sajorcaju* was isolated from fresh fruit body by hyphal method. This edible fungus was grown when the room temperature is between 26-27°C and humidity between 70-85%. It grows upto 5-15cm and it is slightly grey in colour.

3.3.1. MATERIALS USED

1. Spawn bottle (Plate 1)

   The spawn bottle of fungal strain, *Pleurotus sajorcaju* was procured from the Agriculture College and Research Institute of Tamil Nadu Agriculture University, Killikulam, Tirunelveli District.

2. Polythene bags – polythene bags were measuring about the length of 60cms and 30cms width.
3.3.2. METHOD

The straw and maize stalks were chopped with a hard cutter or machine. The chopped straw was about 5cm long. Pods of soyabean, blackgram and greengram were collected without any dust. The chopped paddystraw, waste cotton, pods of soyabean, blackgram and greengram were immersed in water for 6-8 hours in separate containers (Plate 5). After that spreading the wastes on clean platform for an hour drained excess of water out. Then wastes were boiled for complete sterilization and they were allowed to dry again. The wastes with little moisture were ready for packing (Sivaprakasam, 1986).

3.3.3. PREPARATION OF BED

Paddystraw, waste cotton, pods of soyabean, blackgram, greengram and maize stalk were used as bed in the polythene bag. For the preparation of bed, half the amount of spawn bottle was needed for one polythene bag. Again this half amount was divided into four equal parts. First, one end of the polythene bag was tied with twine to prevent the air passage. To start with, the first layer is filled with substrate and the second layer with spawn alternately, so that one polythene bag contains five layers of straw and four layers of spawn. Finally the other end
of the polythene bag was also tied with twine. The bag appeared as a cylinder, 10 to 12 holes were made in the bag for aeration. The bag was kept in the culture room. The same method was also followed for waste cotton, pods of soyabean, blackgram, greengram and maize stalks.

FLOW CHART SHOWING THE STEPWISE PROCEDURE FOR CULTIVATION OF MUSHROOM

Chopped Substrates

Paddy straw, waste cotton, pods of soyabean, blackgram greengram and maize stalk

Soaked in cold water for 6hours

Transferred into hot water (68°C) for about an hour

Dried in shaddy place

Wet substrate mixed with 8% spawn

Filling the substrate into polythene bags in which holes were made for proper aeration

Spawn run at 20-25°C

Fruit body formation (Harvesting)
INTEGRATED SYSTEM OF WASTE RECYCLING (Flow Chart)

Agrowaste

- Mushroom Food from Waste
- Mycowaste
- Waste used as feed for ruminants

- Mycoslurry
- Biogas Energy from Waste
- Animal Excreta waste as feed stock for biogas plant

- Vermicompost Waste as Plant nutrients
- Vermiculture waste as feed for earthworm

Waste to produce food Agriculture field

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Fig. 1. Agro - Industrial Wastes
1.1 Paddy straw
1.2 Waste cotton
1.3 Soyabean pods
1.4 Blackgram pods
1.5 Greengram pods
1.6 Maize stalk

Fig. 2. Spawn (*Pleurotus sajor-caju*) Bottles

Fig. 3. Growth Stages of Mushroom
1 Formation of Mycelium
2 Pin head Stage
3 Button Stage

Fig. 4. Fructification of *Pleurotus sajor-caju*

Fig. 5. Mycosubstrates of Various Wastes
5.1 Mycosoyaben pods
5.2 Mycowastecotton
5.3 Mycopaddystraw
5.4 Mycomaize stalk
5.5 Mycoblackgram pods
5.6 Mycogreengram pods
3.3.4. STAGES OF GROWTH

The place where the mushroom is grown should not be exposed to direct sunlight, rain or wind. After the preparation, packed polythene bag was hung in the culture room, where the temperature of the room is maintained in the range of 26-27°C and the humidity in the range of 70% to 80% which was possible by regular sprinkling of water, preferably twice a day.

After a spawn run period, the mycelium (Plate 1) started spreading on the substrate, after which the pinhead stage appeared within 1-2 days. This is the first stage. After the pinheads, they attained the button stage. Though the holes in the bag, the buttons protrude out of the bag. The young button stage mushroom attained the normal size and developed into beautiful oyster shaped mushroom within 2-3 days. When they are sufficiently large, they were plucked by clockwise twist. This was the first harvest. After the first harvest, the sides of the beds were scrapped with knife for the exposure of inner mycelium. The second crop appeared after 1-2 weeks. Three crops can be harvested within a period of 4-5 weeks. The cultivation period varied widely depending on the type of substrates.
3.4. PHYSICO-CHEMICAL CHARACTERIZATION OF MUSHROOM

3.4.1. TEMPERATURE

Temperature was measured using Parash thermometer. For measuring the culture bag temperature, the thermometer was introduced into the bag and the reading of the mercury level in the thermometer was noted (Saxena and Rai, 1988).

3.4.2. MOISTURE

Weighed out a small quantity (usually 100g) of fresh mushroom sample. Dried in an appropriate oven at 100°C for about 8-24 hours. Weighed sample after drying

\[
\text{Moisture(\%)} = \frac{\text{Loss of weight during drying}}{\text{Weight of sample before drying}} \times 100
\]

(Bano and Rajarathnam, 1988).

3.4.3. pH OF THE SUBSTRATE

The pH of different substrate was determined directly by using the Digital pH meter, ELICO Limited, Hyderabad (Saxena and Rai, 1988).

3.4.4. CHEMICAL CHARACTERISTICS OF MUSHROOM

- Estimation of Protein (Lowry et al., 1951; Plate 5).
- Estimation of Lipids (Joslyn, 1970).
3.5. *Pleurotus sajor-caju* TREATED ORGANIC WASTE AS SUPPLEMENTAL FEED-STOCK FOR BIOGAS DIGESTERS

The possibility of utilizing mycosubstrates as alternative or supplemental feedstock with cowdung for biogas production was studied under laboratory conditions. The mycosubstrates (Plate 1) such as mycostraw, mycocotton, mycosoyabean pods, mycoblackgram pods, mycogreengram pods and mycomaize stalk were collected after the cultivation of mushroom from the cropping room of the college campus. Cowdung was collected from the yards of Sri Parasakthi College, Courtallam, Tenkasi, Tirunelveli District, Tamil Nadu State, India (Map 3:1:1). Biogas slurry was also collected from the Biogas plant at Sri Parasakthi College, Courtallam.

The control and treatments (Plate 3) included in the study were as follows.
1. Cowdung + biodigested slurry ($T_0$)
2. Cowdung + Mycostraw + biodigested slurry ($T_1$)
3. Cowdung + Mycocotott + biodigested slurry ($T_2$)
4. Cowdung + Mycosoyabean pods + biodigested slurry ($T_3$)
5. Cowdung + Mycoblackgram pods + biodigested slurry ($T_4$)
6. Cowdung + Mycogreengram pods + biodigested slurry ($T_5$)
7. Cowdung + Mycomaize stalk + biodigested slurry ($T_6$)

The experimental set up prepared by mixing 90% cowdung mycoslurry with 10% inoculum were poured into 5 litres capacity KVIC (Khadi Village Industry Commission) prototype laboratory model biogas plant (Plate 3) and kept for anaerobic digestion for 30 days. After the completion of 30 days retention period, the charging of substrates (Plate 5) was continued till the end of the experiments.

After the completion of 30 days retention period, every 10 days, 200 gram of digested sample was taken from both control and treatments. These samples were subjected to Total solids, Volatile solids, Organic carbon, Nitrogen, Phosphorus and Potassium analysis.

Temperature was noted daily by using Parash thermomometer (Singh, 1975). pH was noted daily by using pH meter (Chawla, 1986). Gas output was measured by water
displacement method (Diaz et al., 1974; Diaz and Trezek, 1977). All the analyses were continued upto 60 days. Adopting the following methods carried out analysis of the substance.

3.5.1. ANALYTICAL METHODS

- Total solids (APHA, 1975)
- Volatile solids (APHA, 1975)
- Organic carbon (Walkley and Black method, 1965; Plate 5)
- Estimation of Phosphorus (Fiske and Subarao, 1925)
- Estimation of Nitrogen (Jackson, 1958)
- Estimation of Potassium (Jackson, 1958)

3.6 PRODUCTION OF VERMICOMPOST BY USING MYCOBIOGAS SLURRY

Biogas slurry was allowed to decompose in open yards. They cause not only environmental pollution, emanating obnoxious odour, lose its nutrient content due to improper handling and storage but also finally contaminate water bodies. Hence, biogas slurry was mixed with soil and produced the vermicompost. In vermiciculture, beneficial soil bacteriae along with earthworms are the prime force of processing the wastes involved. Earthworms weed out unwanted pathogens. When the earthworms are used to process organic and rock particles in the root zone of plants, they act as bioengineers releasing nutrients in
a balanced manner at the same time improving solids structure by creating crumby soil enriched with macro and micronutrients as well as microorganisms. The locally available species of the earthworm, *Lampito mauritii* (Plate 4) was used in the present study.

3.6.1. SYSTEMATIC POSITION

<table>
<thead>
<tr>
<th>Phylum</th>
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</tr>
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<tr>
<td>Class</td>
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<tr>
<td>Order</td>
<td>- Terricoale</td>
</tr>
<tr>
<td>Family</td>
<td>- Megascolecidae</td>
</tr>
<tr>
<td>Genus</td>
<td>- Lampito</td>
</tr>
<tr>
<td>Species</td>
<td>- Mauritii</td>
</tr>
</tbody>
</table>

*L.mauritii* was collected from the wet area of our college campus. Soil was dug and carefully searched for earthworms. The worms were picked up and placed separately in polythene bags along with a little parent soil. After the collection, the *L.mauritii* were acclimatized in the tray kept in room temperature for about ten days. After acclimatization the healthy and matured earthworms were sorted from the acclimatized tray and they were used for experimental purposes.
3.6.2. CULTURING TECHNIQUE

The circular plastic trough with the height of 12 cm and diameter of 36 cm and thickness of 1 cm was selected for culturing (Plate 4).

3.6.3. CULTURE BED

The bed preparation is an essential feature in the vermitech procedure. The biogas slurry was rich in water content. 50% of biogas slurry was mixed with 50% garden soil and it was used as bed. Ten earthworms per kg of the substrates were added to each plastic trough as per the treatments and incubated for 60 days. Water was added onto the beds to hold 40-50% moisture. The experiment was terminated on the 60th day and the contents of the troughs were, after removing the earthworms, shade dried and sieved through a 2.5mm sieve and analysed for available N, P and K.

3.6.4. EXPERIMENTAL DETAILS OF VERMICOMPOSTING OF MYCOBIOGAS SLURRY (Plate 4)

Replication : Four

Treatments : Eight

Cowdung + Soil + Earthworm (T₀)

Cowdung biogas slurry + Soil + Earthworm (T₁)

Mycostraw biogas slurry + Soil + Earthworm (T₂)

Mycocotton biogas slurry + Soil + Earthworm (T₃)
Mycosoyabean pods biogas slurry + Soil + Earthworm (T₄)
Mycobackgram pods biogas slurry + Soil + Earthworm (T₅)
Mycogreengram pods biogas slurry + Soil + Earthworm (T₆)
Mycomaize stalk biogas slurry + Soil + Earthworm (T₇)

3.6.5. ANALYTICAL METHODS

Determination of Electrical conductivity
(Biswas and Mukherjee, 1993).

Determination of Soil pH (Biswas and Mukherjee, 1993).

Estimation of available nitrogen in soil
(Subbiah and Asija, 1956)

Estimation of available phosphorus in soil
(Olsen et al., 1954)

Estimation of available potassium in soil
(Stanford and English, 1949)

3.7. EFFECT OF VERMICOMPOST ON THE GROWTH OF BHENDI

The present investigations were carried out with an
objective to study the effect of vermicompost on morphological and
yield characteristics in bhendi (Abelmoschus esculentus).

3.7.1. DETAILS OF FIELD EXPERIMENT

The experiment was carried out in the field to study
the influence of vermicompost on the growth and yield of bhendi.
Crop production manual of TNAU (1985) was referred for most
farming activities. The details of the experiment are furnished below.

Design : Randomised Block Design (RBD), Panse and Sukhatme, 1967

Treatments : Eight

Control Compost – (T₀).
Cowdung slurry compost (T₁).
Cowdung mycostraw compost (T₂).
Cowdung mycocotton compost (T₃).
Cowdung mycosoyabean pods compost (T₄).
Cowdung mycobackgram pods compost (T₅).
Cowdung mycogreengram pods compost (T₆).
Cowdung mycomaize stalk compost (T₇).

Plot size : 3.5 x 3 m
Spacing : 60 x 30 cm

3.7.2. MORPHOLOGICAL CHARACTERS

3.7.2.1. PLANT HEIGHT

Five plants selected randomly were measured from the cotyledonary node to the tip of the terminal bud on 25th, 50th and 75th days representing vegetative and flowering stages and mean was worked out and expressed in cm.
3.7.2.2. NUMBER OF LEAVES

The total number of leaves in five plants at each stage was counted and mean was calculated.

3.7.2.3. ROOT LENGTH

The length of the root was measured in five randomly selected plants from the cotyledonary to the tip of the longest root on 25th, 50th and 75th days and mean was expressed in cm.

3.7.3. GROWTH ATTRIBUTES

3.7.3.1. SHOOT ROOT RATIO (S-R RATIO)

From the dry weight of shoot and root the S-R ratio was arrived.

\[
\text{S-R ratio} = \frac{\text{Shoot weight}}{\text{Root weight}}
\]

3.7.3.2 TOTAL DRY MATTER ACCUMULATION (TDMA)

Samples were first sun dried for a week and kept in air oven at 70°C and removed after 24 hours. The dry weights of root, shoot, leaf and whole plant were taken and expressed in g plant\(^{-1}\).

3.7.4. YIELD AND YIELD COMPONENTS

3.7.4.1. DAYS TO FIRST FLOWERING

The days to the first appearance of flower in each treatment from the date of sowing was recorded.
3.7.4.2. FLOWER NUMBER

The total number of flowers produced in five plants in each replication was taken from the first day of flowering to the last day of flowering and the mean was recorded.

3.7.4.3. FRUIT NUMBER

The number of fruits produced in five plants in each replication was recorded and the mean value was also calculated.

3.7.4.4. FRUIT LENGTH

The length of the fruit was measured from the base to the tip of the fruit in five fruits and the mean value was recorded.

3.7.4.5. NUMBER OF SEED PER FRUIT

The total number of seeds per fruit in five plants tagged at random for this purpose in each replication was counted and expressed as seeds per pod.

3.7.4.6. DRY WEIGHT PER POD

Ten fruits at random were taken for recording the fresh weight and were sun dried for drying, weighing and expressed in gram.

3.7.4.7. FERTILITY COEFFICIENT

The daily production of flowers was recorded from first flowering to harvest and the total number of flowers produced per plant was computed. Similarly, the number of pods formed in the
same plant was also recorded and from these data, the percentage of pod set was computed.

\[
\text{Fertility coefficient} = \frac{\text{No. of fruits produced}}{\text{Total No. of flowers per plant}} \times 100
\]

**3.7.4.8. HARVEST INDEX**

Harvest index was calculated by using the following formula and expressed in percentage.

\[
\text{HI (Dry weight basis)} = \frac{\text{Total fruit weight}}{\text{Total plant weight}} \times 100
\]

**3.7.4.9. YIELD PER PLANT**

The total cumulative yield per plot was arrived at from each picking and these values were computed to one hectare and expressed in tones per hectare.

**3.8. BIOSTATISTICAL EVALUATION**

Biostatistical evaluation of the experimental results was carried out with the help of Mean, Standard error and Standard deviation and Analysis of Variance. Least Significant Difference or Critical Difference tested difference in mean for significance. The treatment means were also compared by Student’s ‘t’ test. Correlation co-efficient was used to analyse the relationship among the variables (Snedecor and Cochran, 1968).
Plate 5: Researcher At Work

Fig.1. Processing the greengram pods for the preparation of Mushroom bed.

Fig.2. Charging of the Mycosubstrate with cowdung for Biogas production.

Fig.3. Estimation of Protein.

Fig.4. Estimation of Organic Carbon.