

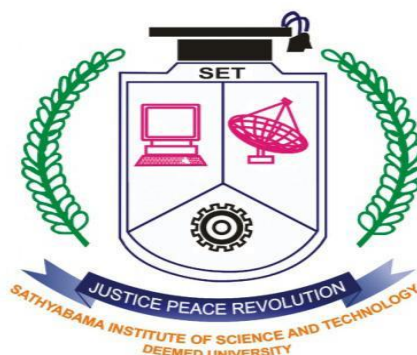
**STUDY OF MORPHOLOGICAL AND PHYTOCHEMICAL VARIATIONS OF
“*OCIMUM SANCTUM*” UNDER DIFFERENT ORGANIC AND INORGANIC
AMENDMENTS**

(Project– Report)

**Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology**

By

K.Rupadharshini (38230027)



DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with 'A' grade by NAAC

JEPPIAAR NAGAR ,RAJIV GANDHI SALAI ,CHENNAI -600119

MAY 2022

BONAFIDE CERTIFICATE

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THE NEW COLLEGE
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PG & RESEARCH DEPARTMENT OF ZOOLOGY



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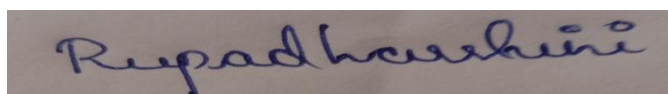
DECLARATION

I, **K.RUPADHARSHINI** hereby declare that the project entitled “**STUDY OF MORPHOLOGICAL AND PHYTOCHEMICAL VARIATIONS OF OCIMUM SANCTUM GROWN UNDER DIFFERENT ORGANIC AND INORGANIC AMENDMENTS**” done by me under the guidance of **DR.Masilamani Selvam (Internal) DR.Rajesh Babu (External)** in the **P.G and Research Department of Zoology ,Chennai -600014.** is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

DATE :

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SIGNATURE OF CANDIDATE :

A rectangular box containing a handwritten signature in blue ink. The signature is written in a cursive style and appears to read 'Rupadharshini'.

ACKNOWLEDGEMENT

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ABSTRACT

Ocimum sanctum , is commonly called as “Tulsi” or “Holy Basil” belonging to the family “Lamiaceae”. The present study is proposed to analyze the potentiality of nutrient rich vermicompost and synthetic fertilizer in the cultivation of medicinally important herb *Ocimum sanctum* under field condition. Organically grown medicinal plants using vermicompost have been found to be highly nutritious, rich in 'proteins, volatiles, minerals and vitamins' and 'antioxidants' than their chemically grown counterparts and can be highly beneficial for human health. The field is chosen and soil fertility test is assessed prior and during the study. Organic wastes were collected locally . Vermicomposting will be carried out in cement tanks using locally available earthworm species . During the cultivation period plant parameters were evaluated by the application of organic and inorganic amendments. Phytochemical screening is done. Volatile profile is analyzed.

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CHAPTER 1 INTRODUCTION

Tulsi (*Ocimum sanctum*) is an omnipresent, multipurpose plant and appeared as holy plant in Hindu faith reveals region in the front of each Hindu household. This herbs belongs to the member of Lamiaceae family, widely known for its spiritual, non secular and medicinal significance in India. *Ocimum sanctum* plant is a small annual or short-lived perennial shrub, up to at least one metre in height. The stems are bushy and endure easy toothed or whole leaves oppositely alongside the stem. The aromatic leaves are inexperienced or crimson, relying at the variety. The small crimson or white tubular flora have inexperienced or crimson sepals and are borne in terminal spikes. The end result are nutlets and convey several seeds.

The plant is respected in Hinduism as a manifestation of the goddess Lakshmi (Tulsi), the foremost consort of the god Vishnu. The plant is grown in lots of Hindu houses, regularly with inside the courtyard in a committed four-sided structure, and the presence of a holy basil plant is assumed to boom piety, foster meditation, purify, and protect. Even the ritual act of watering and worrying for the plant, generally undertaken through the girls of the house, is taken into consideration worshipful and meritorious. Holy basil is cultivated at many temples, and the woody stems of vegetation which have died are used to make beads for sacred japa mala (rosaries). The starting of the Hindu wedding ceremony season is marked through a competition referred to as Tulsi Vivah, wherein houses and temples ceremonially wed holy basil to Vishnu. Water infused with the leaves is regularly given to the death to assist increase their souls, and funeral pyres are normally geared up with holy basil twigs with the hopes that the deceased may also reap moksha and be liberated from the cycle of rebirth.

In the latter half of the twentieth century, scientific research of traditional beliefs about Tulsi's medical benefits gained traction. The plant has a spicy, bitter, hot, light, and dry flavour that indicates the presence of curative properties. Because of its beneficially diversified characteristics, it is well-known and has been utilised in Ayurvedic and Greek

medicine for centuries. The presence of phytochemicals in leaves, such as flavonoids, phenol, terpenoids, alkaloids, eugenol, and others, confers the potential to combat infection by target cells, according to scientific evidence. Antimicrobial, pharmacological, therapeutic, nutritional benefit, or even leveraging of precise quantities of phytochemicals to bring about cellular protection are all examples of abilities.

This review aims to provide a summary of the religious values, physiological aspects, antimicrobial properties, pharmacological application, therapeutic uses, phytochemical analysis, animal nutrition supplement, and biotechnological studies in Tulsi, including molecular marker use, genome sequencing, bioinformatics, and nanotechnology. Alcohols, aldehydes, ethers, esters, hydrocarbons, ketones, lactones, phenols, and phenol ethers are among the organic chemical components found in essential oils, which are complex molecules obtained from plants. Almost all of these organic compounds have been synthesised, and imitation flavourings are made from these synthetics.



FIGURE 1.1



FIGURE 1.2

CHAPTER 2 REVIEW OF LITERATURE

2.1 THE GREEN REVOLUTION IN INDIA

With the advancement of time period and as a result of famine and poverty when the demand for plant based product raised to cope the ever rising population farmers moved to the modern systems of agriculture called Inorganic farming. With the bloom of Green Revolution in the 1960s supported by policies, and fuelled by harsh agrochemicals, machinery and irrigation, are known to have enhanced agricultural production and productivity. *“If farm ecology and economics go wrong, nothing else will go right in agriculture”* stated by M S Swaminathan popularly known as the Father of Green Revolution in India, the missionary of ever-green revolution. His main goal was to eliminate world hunger and poverty especially in India by using environmentally sustainable agriculture, sustainable food security and preservation of biodiversity. He called the preservation of biodiversity as the evergreen revolution. The situation just was just opposite for his thoughts. Even the revolution fueled a selfsufficiency of food for India in 1975. During 1985 India became the food surplus country and with the further advancement of farm practices by chemical additives took a tole on nutrient depletion from soils till date leading to soil hunger. As a result of green revolution it has been estimated that the fertilizer consumption in India raised from ½ kg in 1951-52 to 144 kg till 2009-10 per hectare area. Consumption of NPK fertilizers increased to many thousand tonnes and application rose to six fold though the cost of chemical fertilizer had increased tremendously. In general India gained more power through ‘Green Revolution’ at the cost of Nature.

2.2 EFFECTS OF INORGANIC FARMING

Over usage of chemical fertilizers and chemical additives in agriculture for the past four decades to meet the ever rising human demand led to the complete degradation of soil biota and its naturality in the due course. In the present world environmental degradation is a major threat confronting the world, and the rampant use of chemical fertilizers contribute largely to the deterioration of the environment through depletion of fossil fuels, generation of carbon dioxide (CO₂) and contamination of water resources. Intensive cultivation of land without aim of conservation of soil fertility and

Soil structure led ultimately, to the springing up of deserts. Irrigation without arrangements for drainage resulted in soils getting alkaline or saline. Indiscriminate use of pesticides, fungicides and herbicides caused an adverse effects over biological ecosystem leading to the incidence of life threatening diseases like cancer through the toxic residues present in food and other edible parts. Further, the microbial world of agricultural soil is very much disturbed as a result of application of these high toxic chemical fertilizers. The beneficial microbial consortium depletes as a result of high acidity due to chemical fertilizers. Further, chemical fertilizers get rid of the “Great Engineers” of the soil - Earthworms; soil treated with synthetic fertilizers is lack of earthworms (Ma Brussaard, and Ridder, 1990). Hence the chemical fertilizers applied soil is of poor in aeration, low pH levels, high humic acid and high level of Electrical conductivity providing a stressful environment for plants to propagate. This situation is similar to all crops cultivated. With this green revolution the quality of farm products were diminished in their commercial value and the overall phytochemical aspects. Now there is a growing realization that the adoption of ecological and sustainable farming practices can only reverse the declining trend in the global productivity and environment protection (Wani and Lee, 2002).

2.3 THE BLOOM OF SEOND EVERGREEN REVOLUTION

After the massive success in the initial stages and a complete destruction in the later stages of green revolution M S Swaminathan himeself now coined the term ‘Ever-green revolution’.With the drastic effects faced during the Green revolution, we are now forced to move into a new era of agricultural syatems called as the Evergreen Revolution with an assurance of quality, quantity of plant based products without compromise in the environment. The ultimate aim of this new thrust is to raise agricultural commodities well above the level obtained by the green revolution of 1960s, using technology and regulatory policies more advanced and even safer than those now in existence. With this view as by the statement ‘*old is gold*’ its time to look back and bring our older systems of organic agricultural practices like composting and techniques of biotechnology into existence with some more advanced scientific backup comprising of efficient water management, integrated nutrient supply, soil health care, integrated pest management and efficient post harvest management in existence.

2.4 ORGANIC FARMING

Many things grow in the garden that were never sown there - Thomas Fuller

It is a farming system which supports and strengthens biological processes without recourse to inorganic remedies such as chemicals or chemical additives. Organic agriculture is productive and sustainable (Mader *et al.*, 2002). The most accepted definition of organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. One of the promising methods of organic farming is the use of vermicompost for cultivation practices. It is an eco-friendly approach by which the soil fertility and water-holding capacity is increased and enriches the soil nutrients for sustainable agriculture. Sustainable agriculture refers to the set of practices that conserve the natural motherland without compromising human needs. With this view the study was focused to evaluate the effects of vermicompost prepared from different organic wastes and to compare the efficiency with the currently existing commercial synthetic fertilizers on the phytochemical attributes of *Ocimum sanctum* in open field condition.

2.4.1 ORGANIC FARMING AROUND THE WORLD

Organic agriculture is developing rapidly and today atleast 141 countries produce organic plant products commercially. About one-third of the world's organically managed land almost 11 Metric hectare area is located in the developing countries. Most of this land is in Latin American countries, while Asia and Africa are ranked second and third places, respectively. On a global level, in the year 2008, organic land area increased by almost 1.5 M ha compared to the data for the year 2006. About 28 per cent more land under organic management was reported for Latin America. In Europe, organically managed land increased by 4% and by 27% in Africa (Willer and Klicher, 2009). It has been observed that Austria has the highest percentage (8.40%) of area under organic farming, followed by Switzerland, UK and Germany. From this it is clear almost many countries around the world has been shifted to the natural ecofriendly system of organic farming.

2.4.2 ORGANIC FARMING IN INDIA

In India, only 0.03 per cent of the area is under organic farming, though there is huge scope for bringing more land under organic farming. India has traditionally practised organic agriculture, but the process of modernization, particularly the green revolution technologies, has led to the increased use of chemicals. In recent years, however, limitations of agriculture based on chemical use and intensive irrigation have become apparent and there has been a resurgence of interest in organic agriculture. Renewed interest in organic agriculture is mainly due to two concerns, falling agricultural yield in certain areas as a result of *inter alia* excessive use of chemical inputs, decreased soil fertility and environmental awareness.

The 10th Five-Year Plan encouraged the promotion of organic farming using organic wastes, and integrated pest management (IPM) and integrated nutrient management (INM) practices (GoI, 2008). Even the 9th Five-Year Plan had emphasized the promotion of organic produce in plantation crops, spices and condiments using organic and bio-inputs for the protection of environment and promotion of sustainable agriculture. Presently, many states and private agencies are involved in the promotion of organic farming in India, these also include several ministries and government departments at both central and state levels. The Government of India has also launched the National Programme for Organic Production (NPOP) in the year 2001. The NPOP standards for production and accreditation system have been recognized by the European Commission and Switzerland as equivalent to their country standards. Similarly, the United States Department of Agriculture (USDA) has recognized NPOP conformity assessment procedures of accreditation as equivalent to those in the US. With these recognitions, the Indian organic products duly certified by the accredited certification bodies of India are accepted by the importing countries. Currently, India ranks 33rd in terms of total land under organic cultivation and 88th in agricultural land under organic crops to total farming area.

The states of Uttarakhand and Sikkim have declared their states as 'organic states'. Other states like Maharashtra, Karnataka and Gujarat are practising organic cultivation of chickoo, banana and coconut was found to be more profitable. The major reasons for shift towards organic farming include sustained soil fertility, reduced cost of cultivation, higher

quality of produce, sustained yields, easy availability of farm inputs and reduced attacks of pest and diseases. The Government of Karnataka released a state organic farming policy in 2004. Most of the area in the northeastern states is being used for organic farming. In Nagaland, 3000 hectare area is under organic farming. States like Rajasthan, Tamil Nadu, Kerala, Madhya Pradesh, Himachal Pradesh and Gujarat are promoting organic farming vigorously in both horticultural and floricultural areas (GoI, 2008).

Farmers organizations such as 'Chetana' have been established for the marketing of organic products. This programme was implemented in three states of Andhra Pradesh (Asifabad and Karimnagar), Maharashtra (Vidarbha, Akola and Yavatmal) and Tamil Nadu (Dindigul and Tuticorn).

2.5 VERMICOMPOSTING

Vermicomposting is defined as a natural bio-oxidative and biotechnological process in which detritivorous earthworms interact with microorganisms and other fauna within the decomposer community, accelerating the stabilisation of organic matter and greatly modifying its physical and biochemical properties (Domínguez, 2004). It is emerging as an “environmentally sustainable”, “economically viable” and “socially acceptable” technology all over the world. Vermicomposting is the term given to the process of conversion of biodegradable matter by earthworms into vermicast. In this process, the unavailable nutrients contained in the organic matter are partly converted to more bioavailable forms. The use of earthworms was known for ages as “waste managers” for efficient “composting of food and farm wastes” and as “soil managers” for “fertility improvement” for “farm production”. It is now being more scientifically and also commercially revived. Vermicast is also believed to contain hormones and enzymes which are acquired during the passage of the organic matter through the earthworm gut. The hormones and enzymes are believed to stimulate plant growth and discourage plant pathogens. Overall the vermicast is believed to be an excellent organic fertilizer and soil conditioner. Experiments conducted by Gajalakshmi and Abbasi (2004) confirm the earlier reports that vermicompost has more beneficial impact on plants than the compost. It is one of the easiest methods to recycle organic wastes and to produce quality compost. Earthworms consume biomass and excrete it in digested form called worm casts popularly called as Black gold. The casts are

rich in nutrients, growth promoting substances, beneficial soil micro flora and having properties of inhibiting pathogenic microbes.

2.5.1 EARTHWORM AND SPECIES SUITABLE FOR VERMICOMPOSTING

There are nearly 4,400 species of earthworms have been identified in the world. However, only few of these earthworms are used in vermicomposting. It is generally known that epigeic species have a greater potential as waste decomposers than anecics and endogeics. This is due to predominantly humus consuming surface dwelling nature of the epigeics. The commonly used epigeics are *Eudrilus eugeniae*, *Eisenia foetida* and *Perionyx excavatus* (Kumar 1994) worldwide for vermicomposting.



FIGURE 2.1



FIGURE 2.2

2.5.2 VERMICOMPOST

Vermicompost is the excreta (worm castings) of earthworm, which is a natural rich organic soil amendment formed by the decomposition of organic material by earthworms. It is an odourless, clean, organic material containing adequate quantities of N, P, K and

several micronutrients that are necessary for the growth of plants. Vermicompost is soil improves the structure, texture, aeration and fertility of the soil as well as increases its water-holding capacity. With this phenomena plants grown under such conditions have stronger and deeper root systems aiding in better drought-tolerance and disease resistant. The soil treated with vermicompost has significantly more electrical conductivity (EC) and near neutral pH (Tiwari et al, 1989) which favours plant growth with high productivity. Further the foremost advantage of vermicompost is the presence of increased level of microbial loads, microbial activity, microbial respiration, nitrification, denitrification, enzyme activity and VAM spores. Mucopolysaccharides coated with vermicast acts as an important substrate for aerobic, free-living beneficial microbes. So, cellulolytic, lignolytic, nitrifying and nitrogen-fixing microorganisms were found to present in vermicasts (Satchel, 1983).

2.6 PLANTS AND PHYTOCHEMICALS

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). Phytochemicals are accumulated in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds in varying compositions. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. The use of plants as medicines goes back to early man. Certainly the great civilisations of the ancient Chinese, Indians and North Africans provided written evidence of man's ingenuity in utilising plants for the treatment of a wide variety of diseases.

A group of phytochemicals known as secondary plant metabolites have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property over living systems. Phytomedicine almost went into extinction during the first half of the 21st century due to the use of the 'more powerful and potent synthetic drug'. However, because of the numerous side effects of these drugs, the value of medicinal plants is being rediscovered as some of them have proved to be as effective as synthetic medicines with fewer or no side effects and contraindications. It has been proved that although the effects of natural remedies may seem slower, the results are sometimes better on the long run especially in chronic diseases. Recent research studies have investigated and reported that plant phytochemicals can be used for the treatment of a number of ailments like diabetes, dysentery, diarrhea, cough, asthma, bleeding disorders, bronchitis, fever, AIDS, inflammation, ulcers, malaria, prostate cancer, hypertension, atherosclerosis, hyperlipidemia, male infertility, infant brain ischemia and obesity (Bedi *et al* 2010).

Ocimum sanctum with its rich content of Phenols, Tannins, Flavonoids, Steroids and anthraquinones were also used for medicinal purpose. In ancient medical books several therapeutic effects of basil are stated such as for the treatment of abdominal and chest pain, strengthening the heart, treatment of menstrual bleeding, digestive problems and anti-inflammation are reported. Essential oil from *Ocimum sanctum* is reported to have analgesic, hypnotic, antispasmodic and anti-inflammatory effects. There are more than thousand known and many unknown phytochemicals whose activity is to be investigated intensively and commercialized for public use as medicines for various life threatening diseases

CHAPTER 3 AIM AND SCOPE

3.1 AIM

The present study was aimed to prepare a nutrient rich vermicompost using organic wastes like tea dust, vegetable wastes and press mud using locally available earthworm species *Perionyx excavatus*. The nutrient profile, enzyme activity and microbial load during vermicomposting was studied in detail.

3.2 OBJECTIVES

The present investigations include the following objectives:

- To elucidate the agrotechniques employed for *Ocimum sanctum* cultivation.
- To prepare nutrient rich vermicompost from organic wastes like tea dust, vegetable waste and press mud.
- To analyse the nutrient content of prepared vermicompost prepared from different organic wastes.
- To elucidate the effectiveness of prepared vermicompost and synthetic fertilizers in the physico-chemical parameters of cultivation soil.
- To study the enzyme activity and microbial count during the cultivation period.
- To analyse and compare the phytochemical constituents of leaves of *Ocimum sanctum* cultivated under various amendments.
- To compare the volatile constituents of leaves of *Ocimum sanctum* grown under the influence of different vermicomposts and chemical fertilizer.

CHAPTER 4

4.1 MATERIALS

- Soil sample
- Plant sample
- Organic waste (Tea dust waste ,vegetable waste)
- Distilled water
- Reagents
- GC MS

4.2 METHODOLOGY

4.2.1 AREA SELECTED FOR STUDY

The study area selected for the present study is located in the Gummidipoondi (National Highway NH5) Panchayat union of Thiruvallur District in Tamilnadu.

4.2.2 GEOGRAPHIC DESCRIPTION OF AREA

The site selected for study is a small agricultural city located at 13.41° North latitude, 80.12° East longitude and 17 meters elevation above the Mean Sea Level (MSL) located at a distance of about 42 Km from the heart of Chennai, Tamilnadu, India.

4.2.3 PREPARATION OF LAND FOR CULTIVATION OF “*OCIMUM SANCTUM*”

The land selected for cultivation was well prepared and leveled prior to cultivation. The place selected for cultivation was such that the plants can get proper sunlight for at least 6 hours in a day in every season avoiding shade beneath trees and root competition with those of plants for available nutrients and moisture. Double-dig bed raise method was adopted for the cultivation of *Ocimum sanctum*.

The top soil was removed, mixed and tilled to a depth of about 24” facilitating aeration to roots, good drain and easy penetration of roots deeper into the soil. This was done to remove rocks and debris that could obstruct root growth. Both clockwise and anti-clockwise mode of leveling was done during the preparation of cultivation bed. Pebbles and rocks were handpicked manually. Weeds, grasses and other herbs were removed that colonized the field. The cultivation bed was leveled in such a way to ensure proper drain during rainy season



FIGURE 4.1

4.2.4 PLOT PREPARATION :CULTIVATION BED -FIELD EXPERIMENT DESIGN AND TREATMENT

The experimental plot was a completely randomized block design (CRBD). A plot was designed in the prepared cultivation bed. The size of the study plot was in an area of **1000** sq.ft with equal length and width. The experiments were done in triplicates.

4.2.5 IRRIGATION METHOD

The cultivation bed of *Ocimum sanctum* was irrigated daily.

4.3 PREPARATION OF VERMICOMPOST

Vermicomposting was carried out in cement tanks with dimensions of 5 meters length, 2 meters breadth and 2 meters height were constructed above the ground level beneath the shade of a tree with thatched shed to prevent sunlight.

4.3.1 PREPARATION OF VERMIBED

Vermibed refers to the composting unit of the constructed pit. It was carried out by first placing a basal layer of broken bricks and pebbles followed by coarse sand to a thickness of 6 cm for ensuring proper drainage. This is topped by a layer of coir waste, paddy and coconut husk facilitating retention of water during composting. Next, it is topped by a layer of native garden soil to a height of about 15 cm. The vermibed thus prepared was maintained for moisture by spraying water with a sprayer.

4.4 COLLECTION OF VERMICOMPOST

4.4.1 PRESS MUD

Press mud was collected from Sausan Sugar mills located in Tindivanam of Tamilnadu about 150 km from Chennai, India. The collected press mud was transported to the study site. It was cured to remove sulphur residues by mixing with water and dried in open air conditions for 1 week to remove heat and foul smelling odor.

4.4.2 VEGETABLE WASTE

Vegetable wastes were collected from Koyambedu market located in the heart of Chennai, India. The wastes were transported to the study site.



FIGURE 4.2

4.4.3 TEA DUST WASTE

Tea dust waste was collected from the restaurants and cafeteria located in and around Gummidipoondi area in Tiruvallur district in Tamilnadu, India. It was heaped in open sunlight for 1 week to reduce heat during Vermicomposting (Plate 4).

4.5 PROCESSING OF ORGANIC WASTE

The organic waste thus collected cannot be used for vermicomposting directly as it generates lots of heat. The waste material was heaped in sunlight and exposed to temperatures ranging from 29°C to 32°C for two weeks to get rid of toxic gases generated. To prepare a uniform feed mixture, the wastes was individually mixed with fresh cowdung (urine free) in the ratio of 1:1 on dry weight basis and maintained for 45 – 50 % moisture to initiate microbial activity and break down of complex organic biomolecules into tiny particles. Turning of mixture was given at alternate days manually for uniform decomposition.



FIGURE 4.3



FIGURE 4.4

4.6 PHYSIO –CHEMICAL ANALYSIS OF PREPARED VERMICOMPOST

Physico-chemical analysis was done at regular intervals. Analysis was done from the beginning till the end of the experiment. Samples were collected in a sterile polythene bags from the ongoing vermipits and were taken to the laboratory for complete analysis. Sampling was done at 0th, 30th and 60th day. Analytical grade chemicals supplied by Himedia, Chennai were used for the over all study. All the experiments were done in triplicates and the average values were taken. The analysis was done following standard protocols.

4.6.1 pH (Hanna, 1968)

pH in the sample was measured using an 'Elico' digital meter. The electrode of the pH meter was rinsed in double distilled water and it was standardized using buffer solutions. The samples to be tested were suspended in double distilled water in the ratio of 5:1 (w/v). The suspension was mechanically agitated for 15 minutes and filtered through Whatman No.1 filter paper. The filtrate was measured for pH using the calibrated electrode.

4.6.2 ELECTRICAL CONDUCTIVITY(HANNA , 1968)

Electrical conductivity (EC) in the samples was prepared by mixing 1 gram of sample in 5 ml of sterile double distilled water. The suspension was agitated mechanically in a shaker for 4 hours and filtered using Whatman No.1 filter paper. The filtrate was measured for EC using 'Elico' digital electrical conductivity meters.

4.6.3 ORGANIC CARBON ESTIMATION(JACKSON, 1958)

Organic carbon was estimated in the sample by using the modified calorimetric method of Walkley-Black partial oxidation protocol of Jackson, 1958. 1 gram of sample

from the pit was air dried in a blower and it was powdered and sieved through sieve plate (0.5 mm). The sample was taken in a 500 ml conical flask. 10 ml of N-Potassium dichromate, 20 ml of distilled water and 10 ml of 85% phosphoric acid was added and mixed. Diphenylamine was used as the indicator. The solution was titrated against ferrous sulphate. Appearance of permanent pale pink color is the end point. Again 0.5 ml of N-Potassium dichromate was added with continuous stirring and it was further titrated against more amount of ferrous sulphate until the color of the solution disappeared. Since 1 ml of N-Potassium dichromate corresponds to 3 mg of carbon, the percentage of organic carbon was calculated using the equation,

$$\% \text{ of Organic carbon} = \frac{V_1 - V_2 \times 0.003 \times 100}{W}$$

Where,

V_1 = ml of N-Potassium dichromate

V_2 = ml of N-Ferrous sulphate

W = Weight of the sample

4.6.4 ANALYSIS OF TOTAL KJELDAHL NITROGEN- TKN % (TANDON, 1993)

Total Kjeldahl Nitrogen content in the sample was analyzed by Kjeldahl method stated by Tandon (1993). About 0.5 g of dried sample was transferred to 100 ml Kjeldahl flask. 20 ml of sulphuric salicylic acid mixture was added and allowed to react overnight. After incubation 5 g of sodiumthiosulphate was added and heated gently for 5 minutes. After cooling of the mixture 10 g of sulphate mixture was added and digested over Kjeldahl apparatus at high temperature for 1 hr. when the digestion was completed the mixture was cooled. 10 ml of the cooled digest mixture was transferred into the vacuum jacket of micro-Kjeldahl distillation apparatus. 10 ml of 4% boric acid solution was taken in a conical flask along with few drops of bromicresol green and methyl red indicators. The condenser outlet of the micro-Kjeldahl apparatus is dipped into this conical flask. The funnel of the micro-Kjeldahl apparatus was washed with 2-3 ml of deionized water followed by 10 ml of 40 % NaOH solution. 5 ml of the aliquot was distilled to the flask

containing the boric acid solution. The boric acid solution is further titrated against N/200 H₂SO₄. Blank was also titrated till the same end point as that of the sample.

4.6.5 TOTAL PHOSPHOROUS (TP %)

4.6.5.1 COLOURIMETRIC METHOD (TANDON, 1993)

PRELIMINARY DIACID DIGESTION OF VERMICOMPOST:

Diacid digestion of vermicompost sample was done using HNO₃ and HClO₄ acid in the ratio of 9:4. 10 ml of the prepared diacid was added to the vermicompost sample taken in the flask and mixed properly by gentle swirling. The flask was placed over hot plate at low temperature initially and at high temperatures till the red colored NO₂ fumes ceases. The end point of digestion was confirmed when the liquid becomes colorless. After cooling of the digest 20 ml of deionized distilled water was added in drops and the solution was made up to the mark. The solution was filtered through Whatmann No.1 filter paper.

4.6.5.2 PROCEDURE:

5 ml of aliquot was pipette out to a volumetric flask. 10 ml of vanadomolybdate reagent was added to the flask. The volume was made up with deionized water by complete mixing. The solution was allowed to settle for 30 minutes. On appearance of yellow color absorbance was read at 420 nm with spectrophotometer and the readings were recorded. Total phosphorous content in the sample was determined using the equation,

$$P_2O_5 \% = \frac{\text{Concentration of sample (ppm)}}{\text{Weight of sample}} \times \frac{100}{\text{aliquot of sample}}$$

4.6.6 QUANTIFICATION OF TOTAL POTASSIUM (TK)

Total potassium content of the vermicompost sample was estimated by Flame Photometric method (Tandon, 1993).

4.6.6.1 PREPARATION OF STOCK SOLUTION

1 g of analytical grade KCl was weighed and dissolved in double distilled water in a 1000 ml standard flask and made up to 1 litre. The stock thus prepared contains 1000 ppm of dissolved potassium. From this stock, 10, 20, 30, 40 and 50 ppm standard concentrations of potassium solutions were prepared by appropriate dilutions. \

4.6.6.2 PREPARATION OF STANDAR CURVE

The galvanometer reading was adjusted to 0 in the flame photometer using distilled water. Then, 10, 20, 30, 40 and 50 ppm standard potassium solution was fed into the flame photometer and the corresponding galvanometer reading was recorded. A standard graph was drawn by plotting flame photometer readings on Y- axis and potassium concentrations on X – axis.

4.6.6.3 ANALYSIS OF POTASSIUM OF VERMICOMPOST SAMPLE

The vermicompost sample was atomized in the flame photometer and the galvanometer readings were recorded. The potassium concentration in the sample was determined referring the standard graph and multiplying it with dilution factor.

4.6.7 ESTIMATION OF ZINC AND COPPER

Zinc and Copper content in the vermicompost sample were estimated using Atomic Absorption Spectrophotometer (AAS) as per the protocol stated by Tandon (1993). The AAS readings were read at 213 nm for the estimation of zinc and 324 nm for the estimation of copper in the vermicompost samples. A standard graph was prepared using standard concentrations of zinc and copper (1000 mg/l).

The zinc/copper was determined referring to the standard curve and multiplied with dilution factor.

$$M \times 1000 \text{ ppm.}$$

Where, M is the dilution factor.

4.6.8 ESTIMATION OF CALCIUM

Calcium content in the prepared vermicompost sample was estimated by titration method with EDTA. About 50 grams of dried and sieved sample was taken in a 500 ml conical flask. To this 40 % ethyl alcohol was added. The suspension was shaken for 15 minutes in a rotatory shaker. The aliquot was filtered using a Whatmann No:2 filter paper and the

filtrate was washed for 4 – 5 minutes by 40 % ethyl alcohol. The suspension was mixed with 100 ml of ammonium sulphate and it is incubated overnight. It was filtered again and was used as the extract for Ca estimation. The filtrate was taken in a volumetric flask and was made upto 250 ml with sterile distilled water. From this 10 ml was taken along with 2 ml of NaOH solution and titrated against 0.01 M EDTA solution. Murexide (100 mg) was used as the indicator. Color change from Pink to purple was taken as the end point (Cheng and Bray, 1951).

Calculations:

$$\% \text{ of Calcium} = A \times 400.8 \times V/v \times 1000 \times S$$

Where,

A = Volume of EDTA used (ml)

V = Total volume of extract (500 ml)

S = Weight of the sample taken (50 gm)

V = Volume of extract (ml).

4.6.9 ESTIMATION OF MAGNESIUM IN VERMICOMPOST SAMPLE

50 grams of dried sample was mixed with 100 ml of ammonium acetate in a conical flask. The solution was allowed to leach overnight at room temperature. Further the mixture was filtered using a Whatmann No: 42 filter paper. The filtrate was taken in a volumetric flask and made up to 250 ml with distilled water from which 10 ml was taken in a beaker. To this 2 ml of buffer solution was added and titrated against 0.01 M EDTA. 100 mg of Erichrome black T was used as an indicator. Endpoint is the change in wine red color to blue color (Cheng and Bray., 1951).

Calculations:

$$\% \text{ of Magnesium} = (B - A) \times 400.8 \times V/v \times 1.645 \times 1000 \times S$$

Where,

V = Volume of extract (ml)

S = weight of sample taken (gm)

4.6.10 ESTIMATION OF IRON IN VERMICOMPOST SAMPLE

The iron content in the prepared vermicompost sample was estimated using a spectrophotometer as per the protocol stated by Jackson (1973).

4.6.10.1 PREPARATION OF STANDARD IRON STOCK SOLUTION

0.10 gm of ferrous ammonium sulphate was dissolved in 500 ml of double distilled water in a 1000 ml volumetric flask. 20 ml of 0.6 N HCl was added and made up to 1 litre with double distilled water. The concentration of the solution thus prepared is 100 ppm of iron.

4.6.10.2 PREPARATION OF STANDARD CURVE

A standard curve was drawn using a spectrophotometer reading of the working standard iron solution prepared from the standard stock solution.

4.6.10.3 PROTOCOL

An aliquot of vermicompost sample was digested by dry ashing method. 5 to 60 mg of this content was transferred to 25 ml of volumetric flask. The pH of the test solution was adjusted in the range of 1.5 – 2.7 with dilute Hydrochloric acid followed by the addition of 2 ml of 10 % Hydroxylamine Hydrochloric acid and 1 ml of 1.5 % Orthophanonthroline solution. The solution was made up to 25 ml with distilled water. Solution was measured for absorbance. From the absorbance reading the concentration of Iron in the vermicompost sample were recorded by comparing with Standard Curve.

4.6.10.4 CALCULATION

Concentration of Iron in the vermicompost sample (ppm) = $M \times \text{ppm read on the graph}$. Where, M is the dilution factor.

4.6.11 ESTIMATION OF MANGANESE

The Manganese concentration was estimated using spectrophotometer as per the protocol stated by Tandon (1993)

The sample solution (digested by dry ashing method) was taken in a beaker and evaporated at low temperature over a hot plate till it was dried. To this 5 ml of HNO₃ and 2 ml of Hydrogen Peroxide was added and dried again. On cooling, 2 ml of HNO₃ followed

by 5 ml of Phosphoric acid was added and gently heated till the boiling point. The solution was further cooled and diluted with 10 ml of distilled water.

Further 0.3 g of Potassium Periodate was added. The beaker was covered with watch glass and heated again till the appearance of pink color. 20 ml of distilled water was added with continuous heating for 10 to 15 minutes. The hot solution was cooled and transferred into a 50 ml volumetric flask. The volume was made to 50 ml with proper mixing. The solution was read for absorbance at 540 nm in the spectrophotometer. From the readings the concentration of Manganese in the vermicompost sample was calculated from the Standard Curve.

4.6.11 .1CALCULATION

Concentration of Manganese in vermicompost sample = $M \times \text{ppm read on graph}$

Where, M is the dilution factor.

4.6.12 ESTIMATION OF NITRATE NITROGEN (Stewart *et al.*, 1973).

Nitrate content was estimated by following the protocol stated by Stewart *et al.*, (1973).

4.6.12.1 PREPARATION OF PHENOL DISULPHONIC ACID STOCK

150 ml of concentrated sulphuric acid was mixed with 25 grams weighed volumes of Phenol. After mixing of Phenol it the solution was made upto 225 ml using fuming H_2SO_4 with continuous stirring. It was gently heated for 2 hours in a water bath and stored in amber bottles in refrigerator.

4.6.12.2 PREPARATION OF AMMONIUM HYDROXIDE STOCK SOLUTION

Ammonium hydroxide is mixed with water in the ratio of 1 : 1 in a beaker. 10 mg of sample is mixed with 2 ml of Phenol disulphonic acid. The mixture was further diluted with 10 ml of distilled water. To this mixture ammonium hydroxide solution was added in drops until the pH shoots to 11. The mixture was now filtered through Whatmann No: 1 filter paper and the filtrate were measured for optical density at 420nm. A graph was drawn by using different concentrations of nitrate nitrogen with the same protocol followed for the sample.

4.6.13 ESTIMATION OF NITRITE NITROGEN IN SAMPLE BARNES AND FOLKYARD METHOD (1951).

Nitrite nitrogen estimation in the sample was estimated following the protocol stated by Barnes and Folkhard (1951).

4.6.13.1 PREPARATION OF SULFANILIC STOCK

500 mg of sulfanilic acid (Merck) was weighed and taken in a conical flask. To this 100 ml of 70 % 0.12 M HCL was added and mixed until crystals of sulfanilic acid dissolves. The solution was stored in amber bottles under cold conditions.

4.6.13.2 PREPARATION OF N – (1 NAPHTHYL) – ETHYLENE DIAMINE HYDROCHLORIDE

500 mg of N – (1 naphthyl) – ethylene diamine hydrochloride was weighed and dissolved in 100 ml of HCL in a conical flask and was stored in amber bottles under cold condition.

10 mg of sample was extracted with 5 ml of distilled water. The extract was centrifuged at 3000 rpm for 15 minutes to get a clearer supernatant. The supernatant was mixed with 1 ml sulfanilic acid followed by 1 ml of N – (1 naphthyl) – ethylene diamine hydrochloride and shaken well for proper mixing. The solution was diluted to 25 ml with distilled water and left for 10 minutes. The solution was read for optical density at 520 nm. A standard graph was plotted by using different concentrations of nitrite nitrogen with the same protocol followed for the sample.

4.6.14 ESTIMATION OF TOTAL ORGANIC MATTER CONTENT IN THE VERMICOMPOST SAMPLE

Total organic matter content (TOMC) estimation in the prepared vermicompost sample was done by heating the sample at 540 ° C in an electric oven for a duration of 16 hours. The loss in weight of the sample gives the TOMC value (Navarro et al., 1990).

4.6.15 ESTIMATION OF TOTAL CARBON CONTENT AND OXIDISABLE CARBON CONTENT

Total carbon content Oxidisable carbon content in the vermicompost sample was estimated by the expression stated by Navarro et al., (1990). Which states,

Percentage of Total carbon content (% TCC) = (TOMC – 9.33)/1.745

Percentage of Organic carbon content (% OCC) = (TOMC – 15.356)/1805

pH		5.72±0.31
Electrical conductivity (dSm ⁻¹)		0.025±0.006
Cation exchange capacity %		4.45±0.11
Organic matter (%)		4.23±1.02
Total Kjeldahl Nitrogen (%)		4.23±0.11
Phosphorous (ppm)	Bray 1	211±1.33
	Bray 2	265±1.00
Potassium (%)		5.45±0.24
Calcium (%)		222±1.64
Magnesium (%)		57.4±1.98
Sulphur (ppm)		10.23±0.06
Sodium (ppm)		17.65±1.47
Zinc (ppm)		0.078±0.007
Manganese (ppm)		6.85±0.03
Iron (ppm)		9.87±0.45
Copper (ppm)		0.63±0.11
Boron (ppm)		0.45±0.35
K saturation		0.36±2.21
Ca saturation		39.87±0.25
Mg saturation		6.57±0.08
Na saturation		1.25±0.75

TABLE 4.1

4.7 SOIL FERTILITY ANALYSIS

FERTILITY STATUS (PHYSICO- CHEMICAL ANALYSIS) OF THE CULTIVATION BED FOLLOWING THE AMENDMENTS OF ORGANIC AND INORGANIC FERTILIZERS

In order to fulfill the main objective of the study i.e., monitoring the soil fertility status of the study plot was assessed by studying the physical, chemical and biological properties prior to study (control plot) and also during the course of cultivation.

4.7.1 OVERALL INPUT OF ORGANIC AND INORGANIC AMENDMENTS USED IN THE PRESENT STUDY

The various organic and inorganic amendments were given in split doses. All the necessary cultural practices and protective measures were followed uniformly for all the respective treatments during the entire period of study. Organic amendments were applied at the rate of 6 tons ha⁻¹. Inorganic amendment was applied at the rate of 14 tons ha⁻¹ (standard dosage of fertilizer used for *Ocimum sanctum* cultivation).

4.7.2 SOIL SAMPLING

Soil sampling was carried out following a standard method (Jackson, 1973). For this, samples from the cultivation bed were collected by digging pits between each plant at three different depths i.e. from 0 to 15 cm (surface), 15 to 30 cm (middle) and 30 to 60 cm (lower) and scraping the walls from all sides at each depth. The process was repeated at 4-5 locations for each treatment and the samples were thoroughly mixed on a newspaper. After dividing the samples into four equal parts, the two opposite parts were rejected and the other two parts were mixed again. By repeating the process, 250g of soil samples were taken for analysis. The top soil (0 – 22 cm) was considered for analyzing the physical properties and 15 to 30 cm depth soil was assessed for soil micro flora. The soil was analyzed for pH, Electrical conductivity, organic matter, total Kjeldahl nitrogen, phosphorous, potassium, calcium, magnesium, sulphur, zinc and micronutrients like manganese, iron, copper, Boron and saturations of potassium, calcium, magnesium and sodium.



FIGURE 4.5



FIGURE 4.6

4.8 DETERMINATION OF SOIL PH

The pH of the soil was tested following standard protocols of Jackson (1973).

4.8.1 DETERMINATION OF ELECTRICAL CONDUCTIVITY

The electrical conductivity was measured as per the protocols of Jackson (1973).

4.8.2 ESTIMATION OF ORGANIC MATTER

The total organic matter content of the soil samples were analyzed by through the protocols of Walkley and Black (1934).

4.8.3 ESTIMATION OF TOTAL KJELDAHL NITROGEN

Total Kjeldahl nitrogen content of the soil sample was estimated by Kjeldahl method as described by Tandon (1993).

4.8.4 ESTIMATION OF CATION EXCHANGE CAPACITY

Estimation of cation exchange capacity of soil is done following the protocols of Toth and Prince (1949).

4.8.5 ESTIMATION OF AVAILABLE PHOSPHOROUS

Available phosphorous was estimated by the methods of Olsen *et al.* (1954).

4.8.6 ESTIMATION OF AVAILABLE POTASSIUM

Level of potassium in the soil sample was estimated by the methods of Tandon (1993).

4.8.7 ESTIMATION OF EXCHANGEABLE CALCIUM AND MAGNESIUM

Level of exchangeable calcium and magnesium in the soil sample is determined by the protocols stated by Cheng and Bray (1951).

4.8.8 ESTIMATION OF EXCHANGEABLE SULPHUR

The level of exchangeable sulphur is determined following the protocols of Chesnin *et al.* (1950).

4.8.9 ESTIMATION OF ZINC, IRON, MANGANESE AND COPPER

Level of zinc, iron, manganese and copper was determined following the protocols advocated by Lindsay and Norvell (1978).

4.8.10 DETERMINATION OF BORON LEVEL

Determination of available boron is done following the protocol stated by Berger and Truog (1939).

4.8.11 SATURATION LEVELS OF POTASSIUM, CALCIUM, MAGNESIUM AND SODIUM

Saturation levels in the soil samples were analyzed by the protocol of Tandon (1993).

	CONTROL		TDVP	PMVP	VWVP	CFP
pH	5.72±0.31		5.35±0.19	5.89±0.15	6.02±0.58	5.11±0.24
Electrical conductivity (dSm ⁻¹)	0.025±0.006		0.036±0.010	0.059±0.009	0.048±0.015	0.003±0.001
Cation exchange capacity %	4.45±0.11		6.35±0.19	8.87±0.03	11.2±0.17	1.78±0.09
Organic matter (%)	4.23±1.02		5.84±0.09	6.35±0.98	8.97±1.37	0.99±0.59
Total Kjeldahl Nitrogen (%)	2.35±0.11		7.17±0.05	11.24±0.23	15.42±0.89	2.87±0.11
Phosphorous (ppm)	Bray 1	232±1.33	156±0.22	179±0.54	163±1.28	189±0.45
	Bray 2	214±1.00	147±0.05	161±0.08	158±0.11	238±0.17
Potassium	5.45±0.24		11.45±0.09	21.32±0.74	18.45±0.89	4.65±0.27

(%)					
Calcium (%)	98±1.64	112±0.29	136±0.54	159±0.98	145.3±0.51
Magnesium (%)	15.4±1.98	63.7±0.18	48.3±0.47	79.3±1.23	38.7±0.98
Sulphur (ppm)	2.23±0.06	4.65±0.55	3.11±0.25	5.11±0.25	19.67±0.48
Sodium (ppm)	9.65±0.47	14.19±0.32	19.24±0.23	12.24±0.57	58.24±0.89
Zinc (ppm)	0.078±0.007	0.045±0.003	0.031±0.005	0.031±0.001	0.003±0.005
Manganese (ppm)	6.85±0.03	4.58±0.11	5.23±1.89	3.89±0.19	0.84±1.89
Iron (ppm)	4.87±0.45	11.23±0.09	9.45±0.57	13.45±0.84	6.53±0.11
Copper (ppm)	0.63±0.11	1.24±0.81	4.36±1.54	2.94±1.02	4.99±0.21
Boron (ppm)	0.45±0.35	0.17±0.63	0.58±0.12	0.87±1.09	0.23±1.09
K saturation	0.36±2.21	1.02±1.04	0.76±0.57	1.23±0.99	0.51±0.31
Ca saturation	6.87±0.25	14.43±0.12	21.23±0.98	18.54±0.17	89.35±0.45
Mg saturation	2.57±0.08	4.28±1.24	7.53±0.75	5.41±0.35	1.99±0.19
Na saturation	1.25±0.75	0.98±0.09	1.02±0.11	0.25±0.12	11.87±0.58

TABLE 4. 2

4.9 ANALYSIS OF ENZYME ACTIVITY IN THE CULTIVATION SOIL SAMPLE

Analysis of enzymatic activities prior and during the cultivation period (soil) were done at regular intervals. Activity was measured for Indole acetic acid (Woheler, 1997), Dehydrogenase (Garcia *et al.*, 1997), Acid and Alkaline Phosphatases (Garcia *et al.*, 1997), Urease (Kandeler and Garber, 1988) and Catalase (Rodriguez-Kabana and Truelove (1982) enzyme activity.

4.9.1 ANALYSIS OF INDOLE-3-ACETIC ACID (IAA) IN THE SOIL SAMPLE

Indole-3-acetic acid study in the soil sample was studied by the using the protocol stated by Woheler, 1997. About 2 grams of fresh sample were scooped from the

vermicomposting pit and was collected in a 50 ml centrifuge flask. To this mixture 6 ml of 1% glucose prepared in phosphate buffer (pH 7.5) and 4ml of 1% L-tryptophan were added and mixed. The mixture was incubated at 37 °c for 24 hours in dark condition. After incubation 2 ml of L-tryptophan was added to the controls. 2 ml of 5% trichloroacetic acid solution followed by 1 ml of 0.5 M CaCl₂ solution was added to inactivate the active enzymes. The aliquot was centrifuged at 5000 rpm for 5 minutes. About 3 ml of the supernatant was taken in a test tube. To this 2 ml of salpers's solution (2 ml of 0.5 M FeCl₃ and 98 ml of 35 % perchloric acid) were added. The mixture was incubated at 25°C for 30 minutes in dark. After incubation the red colored solution was measured for absorbance in a spectrophotometer at 535 nm. The same test was done for the control pit.

Sample	Days						
	0	10	20	30	40	50	60
CP	0.15±0.21	0.83±0.11	1.16±0.35	2.18±0.17	2.66±0.64	3.85±0.09	2.83±0.13
TDVC	3.94±0.23	6.03±0.77	6.98±0.01	9.45±1.23	11.24±0.23	12.13±0.35	10.14±0.69
PMVC	4.39±0.11	6.78±0.23	10.97±0.03	16.45±0.23	15.11±0.28	18.72±0.89	16.35±0.78
VWVC	3.06±0.21	4.91±0.36	6.89±0.24	6.26±0.49	8.57±0.26	10.36±0.91	9.14±0.47
CFP	0.65±0.53	1.56±0.87	0.09±0.07	0.05±0.91	0.01±0.57	0.47±0.62	0.01.83±0.98

TABLE 4.2

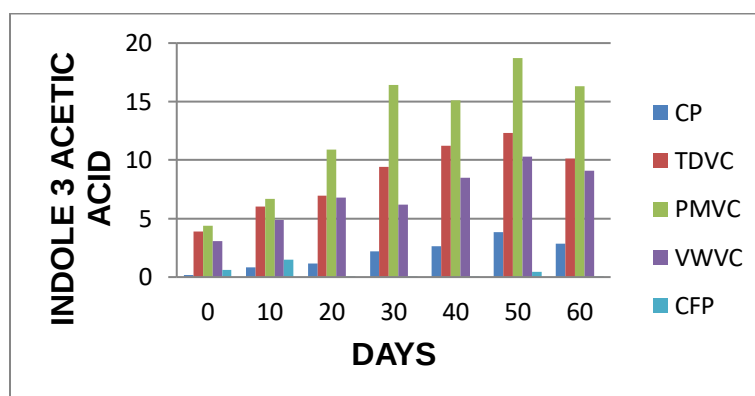


FIGURE 4.7

4.9.2 ANALYSIS OF DEHYDROGENASE ACTIVITY IN THE SOIL SAMPLE

Dehydrogenase activity in the soil sample was described following the protocol stated by Garcia *et al.*, (1997).

4.9.2.1 PREPARATION OF 3% (W/V) 2,3,5-TRIPHENYL TETRAZOLIUM CHLORIDE (TTC) STOCK SOLUTION :

300 mg of of TTC and 0.6057 grams tris- (hydroxylmethyl) methylamine were weighed and taken in a 50 ml conical flask. It was dissolved in 10 ml deionized water. The solution was prepared fresh before use and kept in dark until use.

4.9.2.2 METHANOL STOCK SOLUTION

10 mg 2, 3, 5-triphenyl tetrazolium formazan (TPF) was dissolved in 1 ml methanol. The solution is stored below 4°C until further use. Working standards were prepared by diluting the stock solution with methanol to give TPF concentrations in the range 2 to 8µg TPF/ml.

About 1g of Sample from the vermicomposting and control pits was weighed in weighing balance and was taken in screw cap bottles. To the sample 0.2ml TTC solution and 0.5 ml deionized water was added with mild agitation. The mixture was well mixed and was incubated under dark condition at 30°C for 8 hours duration. The incubated mixture was further added with 10 ml of methanol and shaken by placing over a shaker for 30 minutes. The aliquot was further centrifuged at 2000 rpm for 30 minutes. The supernatant was collected in a separate volumetric flask and made up to 25 ml with methanol. The solutions were allowed to stand and the optical densities were read at 485 nm in a spectrophotometer. Methanol was used as the blank.

4.9.2.3 CALCULATION

Results were obtained in volumes of hydrogen transferred during the reduction of 2, 3, 5-triphenyl tetrazolium chloride (TTC) to 2, 3, 5-triphenyl tetrazolium formazan (TPF) in 1 gram of dry sample. The dehydrogenase activity was calculated by the formulae,

Dehydrogenase activity = $\frac{(S-B) \times F \times 25 \times 0.15035}{\text{Sample weight}}$

W

Where,

S – Height of the Peak

B – Baseline Height

F – Slope of the calibration graph

W – Weight of the sample (g)

Analysis of Dehydrogenase activity was done at every 10 days of duration.

Sample	Days						
	0	10	20	30	40	50	60
CP	1.32±0.23	1.38±0.22	1.48±0.20	1.59±0.17	1.84±0.04	1.96±0.03	2.06±0.07
TDVC	6.35±0.25	7.76±0.11	8.39±0.54	10.11±1.05	14.97±0.57	18.23±0.09	14.27±0.87
PMVC	6.03±0.14	9.13±0.24	10.57±0.63	12.04±0.35	15.35±0.23	17.68±0.17	13.24±0.28
VWVC	5.26±0.07	5.57±0.36	7.70±0.89	8.25±0.59	12.54±0.87	12.33±0.39	14.52±0.01
CFP	0.09±0.21	0.14±0.27	0.03±0.17	0.10±0.63	0.02±0.14	0.39±0.64	0.21±0.98

TABLE 4.3

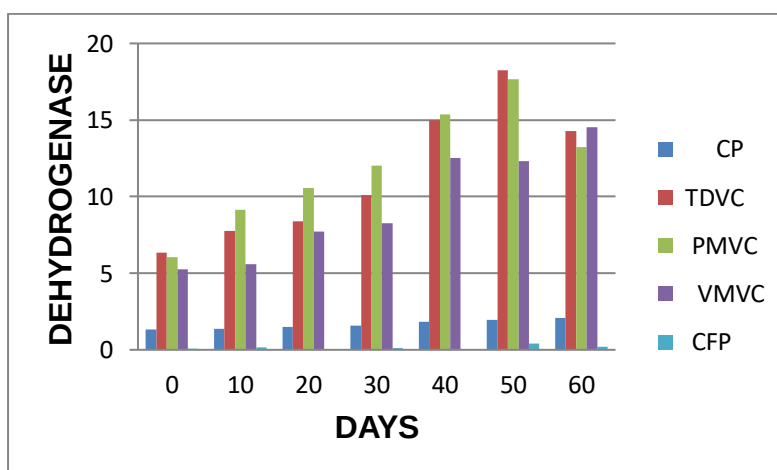


FIGURE 4.8

4.9.3 ANALYSIS OF ACID AND ALKALINE PHOSPHATASES ACTIVITY IN THE SOIL SAMPLE

Acid and alkaline Phosphatases activity in the soil sample was estimated by the method stated by Garcia *et al.* (1997).

Acid and alkaline phosphatases were assayed by picking 1 g of fresh sample from the composting pit. The sample was taken in a clean test tube. To this sample 4 ml of 0.1 M maleate buffer (pH 6.5) was added followed by 1 ml of 25 mM p-nitro phenyl phosphate solution (chromogenic substrate). The mixture was allowed to react for 1 hour at 37 ° C in a water bath. The reaction was terminated by keeping on ice and adding 4 ml of 0.5 M NaOH and 1 ml of 0.5 M CaCl₂. The mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was collected and the absorbance was read at 405 nm. Enzyme activity was expressed as 1 gram p-nitro phenol released g⁻¹ soil h⁻¹ . Phosphatases activity was done at every 10 days of duration.

Sample	Days						
	0	10	20	30	40	50	60
CP	0.12±0.12	1.54±0.23	1.98±1.21	1.59±0.67	3.45±0.14	3.24±0.19	2.64±0.54
TDVC	3.24±0.87	4.87±0.15	7.54±0.15	10.12±0.91	14.51±0.29	17.23±0.02	24.03±0.87
PMVC	12.23±0.81	12.99±0.24	14.12±0.23	15.24±0.26	17.25±0.88	19.14±0.23	21.03±0.54
VWVC	11.51±0.41	11.72±0.78	12.03±0.12	14.59±0.24	14.99±0.32	17.01±0.23	18.11±0.54
CFP	0.53±0.98	0.99±0.56	1.27±0.91	0.87±0.54	1.01±0.66	0.67±0.54	0.05±0.11

TABLE 4.5

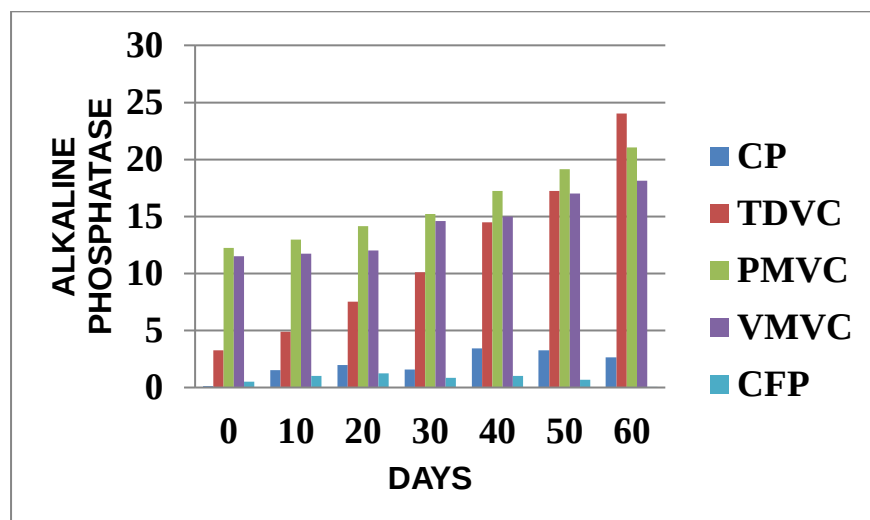


FIGURE 4.9

Sample	Days						
	0	10	20	30	40	50	60
CP	0.18±1.03	0.57±0.89	0.23±0.75	0.59±0.23	0.89±0.13	2.17±0.26	1.98±0.56
TDVC	0.27±0.87	0.69±0.53	1.12±0.87	1.49±1.93	1.88±0.02	2.47±0.69	2.23±0.86
PMVC	2.41±0.04	2.95±0.57	2.36±1.03	3.15±1.56	3.89±0.23	4.43±1.02	6.11±0.12
VWVC	0.19±0.84	1.56±0.18	1.99±0.11	2.03±0.58	3.89±1.02	4.56±0.21	4.04±0.02
CFP	0.51±0.41	0.02±0.09	0.32±0.14	0.07±0.05	0.17±0.12	0.98±0.12	0.32±0.21

TABLE 4.4

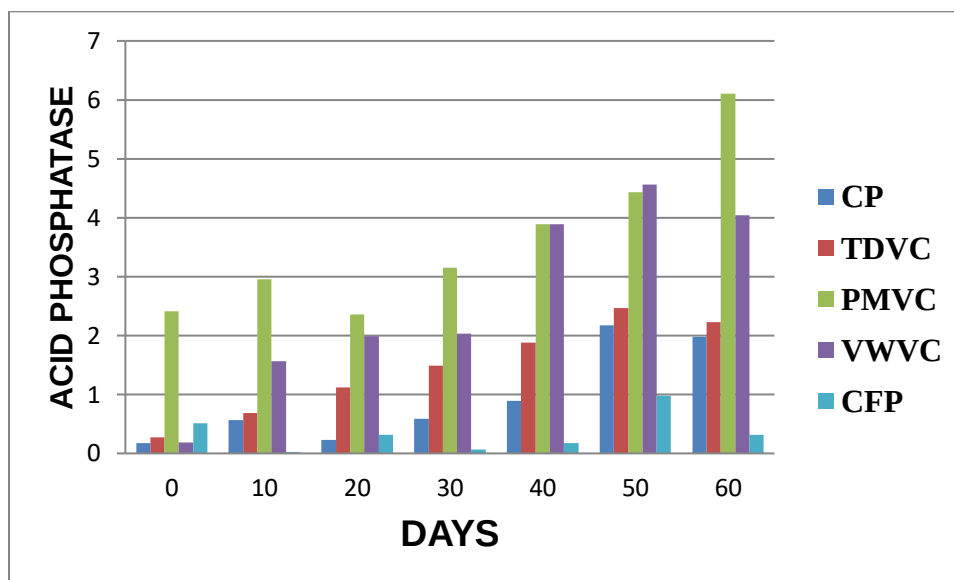


FIGURE 4.10

4.9.4 ANALYSIS OF UREASE ACTIVITY IN THE SOIL SAMPLE

Urease activity in the soil sample was analyzed as per the protocol stated by Kandeler and Gerber (1988).

2.5 ml of urea (80mM) solution and 20 ml of 75 mM borate buffer (pH 10.0) was prepared and taken in a conical flask. 5 g of vermicompost sample was added to the flask and the mixture was made to react for 4 hours placing over a mechanical shaker. Controls were prepared by adding 2.5 ml of sterile double distilled water and 20 ml of borate buffer. After 4 hours of incubation time 2.5 ml of sterile double distilled water was added to the mixture. 2.5 ml of urea was added to the controls. It is then extracted with 30 ml acidified 2 M KCL. The flask was again placed over the mechanical shaker for 30 minutes. From this 1.5 ml aliquots were taken in a centrifuge tube and were centrifuged at 5000 rpm for 5 minutes. 1 ml supernatant was collected and mixed with 9 ml of sterile double distilled water, 5 ml of sodium salicylate and 2 ml of dichloroisocyanuric acid (Na^+ salt). The suspension was incubated at $20 \pm 2^\circ\text{C}$ for 1 hour. The color intensity was measured at 690 nm using a spectrophotometer. Ammonium concentrations were calculated using a calibration curve of standard ammonium chloride solutions from 0 to $2.5 \mu\text{g ml}^{-1}$. Urease activity was studied every 10 days of duration.

Sample	Days						
	0	10	20	30	40	50	60
CP	0.35±0.36	1.23±0.09	1.45±0.98	0.45±0.85	2.24±0.68	2.05±0.87	1.38±0.18
TDVC	2.45±0.56	2.54±1.51	3.42±0.11	3.05±0.29	4.80±1.26	7.38±1.43	5.36±0.42
PMVC	5.05±0.39	5.55±1.57	7.30±0.69	7.63±0.69	10.59±0.10	11.26±0.74	6.37±0.35
VWVC	3.89±0.21	4.08±0.87	7.59±0.04	6.92±0.39	6.26±1.89	7.50±1.49	5.32±0.21
CFP	0.06±0.18	0.07±0.01	0.11±0.10	0.05±0.21	0.01±0.52	0.18±0.23	0.59±0.57

TABLE 4. 5

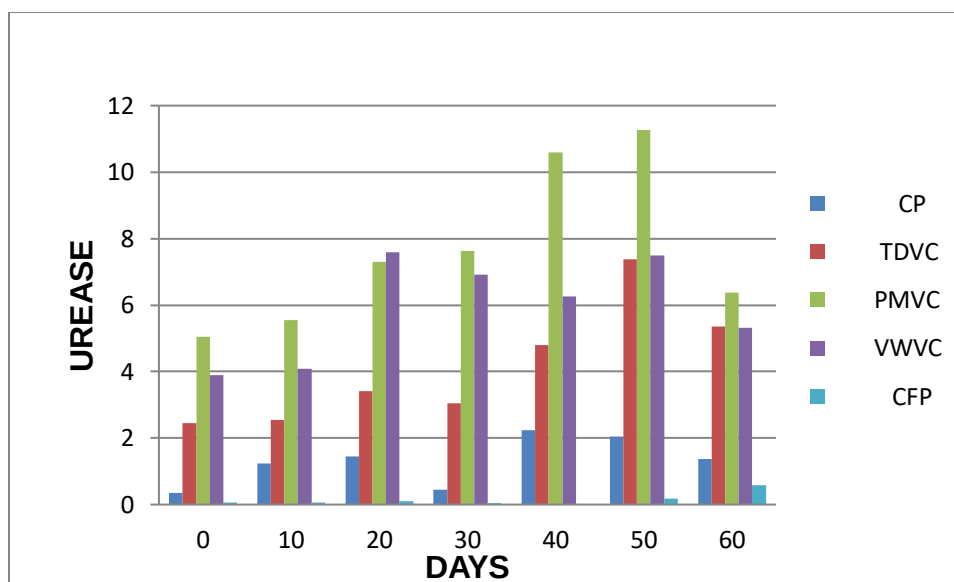


FIGURE 4.11

4.9.5 ANALYSIS OF CATALASE ACTIVITY IN THE SOIL SAMPLE

Catalase activity in the soil sample was determined following the protocol stated by Rodriguez-Kabana and Truelove (1982).

0.5 g of sample was added to 40 ml of distilled water and 5 ml of 30 % H₂O₂ in a conical flask and shaken well for 15 minutes for proper mixing. To this solution 5 ml of 3 N H₂SO₄ was added in drops. The solution was filtered through Whatmann No:1 filter paper and the filtrate was titrated against 0.05 N KMNO₄. Samples without the addition of H₂O₂ are treated as the controls. Catalase activity analysis was done once in 10 days.

Sample	Days						
	0	10	20	30	40	50	60
CP	0.11±0.53	0.81±1.11	1.47±0.54	1.98±0.04	2.54±0.19	3.02±0.98	4.23±0.24
TDVC	3.87±0.14	4.02±0.26	4.89±0.23	5.21±0.23	7.41±0.57	10.98±0.54	9.58±0.23
PMVC	5.21±0.23	5.74±0.21	6.45±0.23	8.19±0.54	10.23±0.57	11.89±0.21	14.54±0.53
VWVC	2.89±0.15	4.57±0.36	6.59±0.35	10.26±0.57	9.53±0.57	12.54±0.89	17.89±0.97
CFP	0.91±1.02	1.23±0.07	0.67±0.54	0.01±0.34	0.87±0.12	1.57±0.34	0.37±0.47

TABLE 4.6

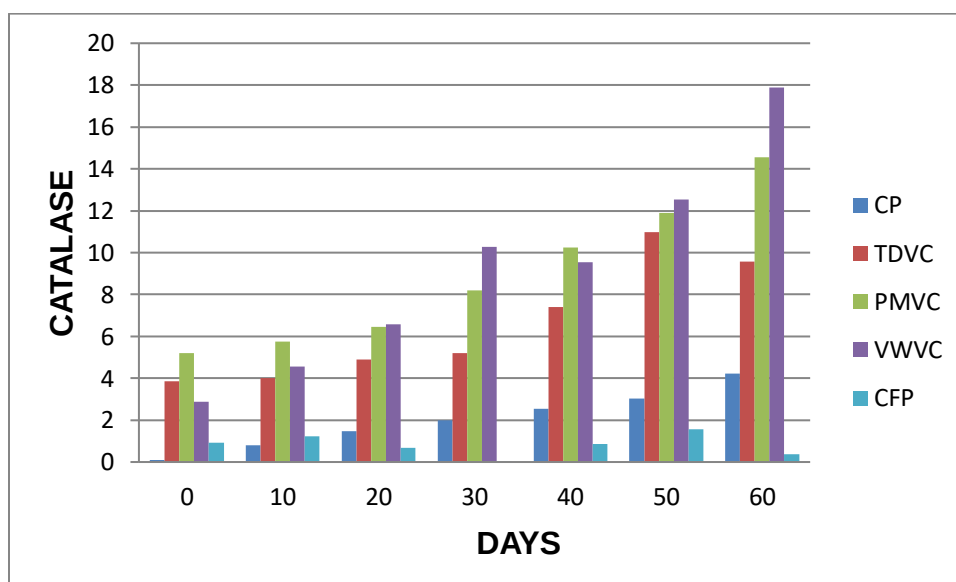


FIGURE 4.12

4.10 ANALYSIS OF MICROBIAL LOAD IN THE SOIL SAMPLE

4.10.1 ESTIMATION OF TOTAL POPULATION OF BACTERIA AND FUNGI

Microbial load in the soil sample was carried at regular intervals from the beginning till the end of the experiment following the protocol stated by Baruah and Barthakur, 1997 for bacteria and Aneja, 1996 for fungi.

4.10.2 NUTRIENT AGAR MEDIUM FOR THE ESTIMATION OF TOTAL BACTERIA

PREPARATION OF NUTRIENT AGAR MEDIUM (BARUAH AND BARTHAKUR, 1997)

1. Beef extract (water soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts) – 3.0g
2. Peptone (amino acids and long-chained peptides) – 5.0 g
3. Sodium chloride – 5.0 g
4. Agar (solidifying agent) – 15.0 g
5. Water – 1000 ml.
6. pH – 7.2

The components mentioned above were weighed and dissolved in 1000 ml of sterile double distilled water in a conical flask. The conical flask was autoclaved at 121 ° C, 15 lb pressure for 15 minutes and cooled to 50°C. 20 ml of this medium was poured to autoclaved petridishes and allowed for solidification. The plates are again autoclaved at 121 ° C, 15 lb pressure for 15 minutes prior to study

Sample	Days						
	0	10	20	30	40	50	60
CP	1.23±0.27	1.98±0.56	2.57±0.45	2.89±0.63	3.51±0.59	5.11±0.27	3.69±0.63
TDVC	5.41±0.57	5.13±0.19	6.98±0.01	8.63±0.17	10.59±0.14	7.23±0.17	8.56±0.12
PMVC	5.07±0.37	6.12±0.89	6.78±0.17	9.21±0.47	10.69±0.48	9.04±0.98	9.21±0.89
VWVC	4.59±0.12	5.63±0.27	6.98±0.47	9.28±0.69	9.07±0.59	9.89±0.68	7.03±0.57
CFP	1.14±0.53	1.33±0.10	1.21±0.87	0.51±0.31	0.47±0.01	0.30±0.21	0.63±0.01

TABLE 4. 7

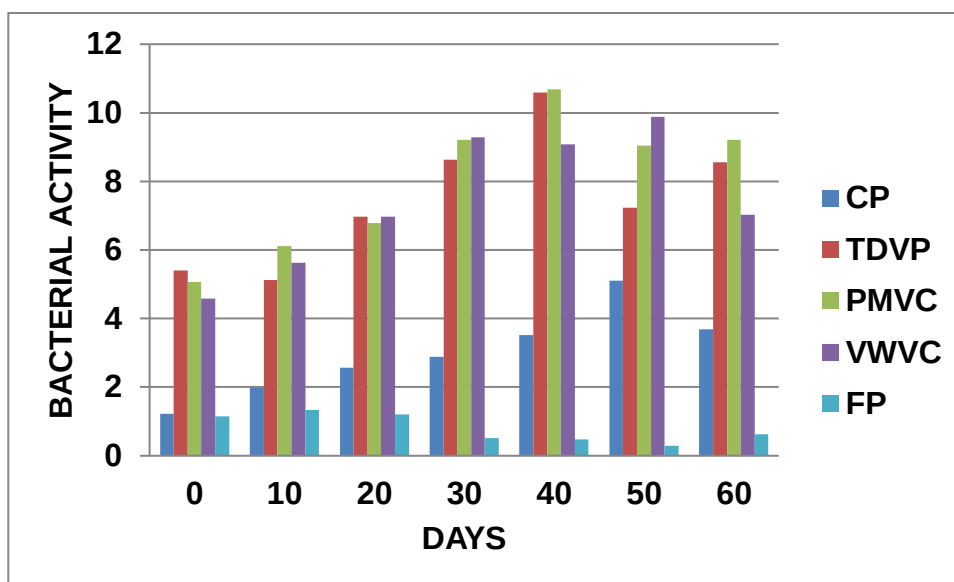


FIGURE 4.13

4.10.3 GROWTH MEDIUM FOR THE ESTIMATION OF TOTAL FUNGI IN THE PREPARED VERICOMPOST

MARTIN'S ROSE BENGAL – STREPTOMYCIN AGAR MEDIUM (RBA MEDIUM) (ANEJA, 1996)

COMPOSITION

1. Soy peptone (carbon and nitrogen source) – 5.0 g
2. Dextrose (energy source) – 10.0 g
3. Potassium dihydrogen phosphate (buffer) – 1.0 g
4. Magnesium sulphate (trace elements) – 0.5 g
5. Rose Bengal (bacterial growth regulator) – 0.05 g
6. Streptomycin (antibiotic) – 0.30 g
7. Agar (solidifying agent) – 15.0 g
8. Distilled water – 1000 ml.
9. pH – 7.2

32.0 g of the medium was dissolved in 1000 ml of sterile distilled water in a conical flask. It was autoclaved at 121°C, 15 lb pressure for 15 minutes. The medium was cooled to 45°C. The antibiotic streptomycin was sterilized separately and was added to the sterile medium. 20 ml of medium was poured to sterile petri dishes and allowed to solidify. The Petri dishes were again autoclaved at 121°C, 15 lb pressure for 15 minutes prior to study.

4.10.4 PROTOCOL

1 g of weighed sample taken from each pit was suspended in 10 ml of sterile double distilled water. The mixture was shaken 5 minutes for proper mixing. Soil particles in the mixture were allowed to settle at the bottom of the container. From this 1 ml of the solution was drawn aseptically into a test tube containing 9 ml of sterile double distilled water to give a 10 fold dilution. Serial dilutions were prepared from this suspension to obtain 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} suspensions.

1 ml of aliquot was pipetted out from each dilution and was aseptically poured onto the sterile petridishes containing Nutrient Agar and Martin's Rose Bengal medium. The aliquot was dispersed over the medium by gently rotating the petridish. The inoculated plates were incubated for 24 to 72 hours at 37°C for Nutrient Agar and at 25°C for Martin's Rose Bengal agar. All the experiments were carried out in triplicates. Plates without inoculums were taken as the control.

After incubation the petriplates were studied for colony counts. The number of bacterial and fungal colonies appeared over the medium in the petridishes were counted and recorded. The colony morphology for bacteria and fungi were studied using high power binocular microscope (Canon). The observations were compared and studied with the monographs by Domsch *et al.* (1980).

Sample	Days						
	0	10	20	30	40	50	60
CP	1.27±0.57	1.98±0.57	2.11±0.21	3.87±0.58	4.35±0.84	3.27±0.47	2.65±0.57
TDVC	4.57±0.28	5.59±0.38	7.03±0.11	8.56±0.64	6.22±0.56	8.10±0.59	6.99±0.14
PMVC	6.23±0.25	6.99±0.87	7.59±0.99	7.35±0.27	8.13±0.22	10.24±0.58	10.74±0.26
VWVC	5.56±0.24	7.63±0.36	6.19±0.17	7.83±0.23	6.53±0.38	9.24±0.26	7.54±0.01
CFP	2.93±0.07	2.54±0.89	1.98±0.32	1.24±0.88	0.99±0.94	0.58±0.14	0.23±0.87

TABLE 4.8

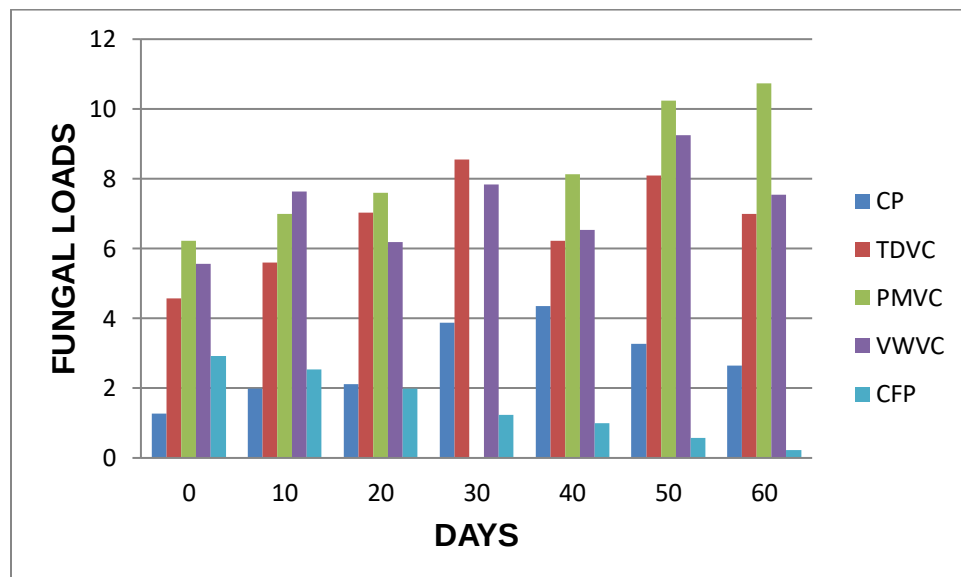


FIGURE 4.14

4.11. PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAVES OF *OCIMUM SANCTUM* GROWN UNDER ORGANIC AND INORGANIC AMENDMENTS

4.11.1 QUALITATIVE PHYTOCHEMICAL SCREENING OF LEAVES OF *OCIMUM SANCTUM*

4.11.1.2 COLLECTION OF LEAF MATERIAL

For phytochemical analysis fresh leaves from the treated plots were plucked in the early hours of dawn and was collected in wet cloth bags and carried to the laboratory for further studies.

4.11.1.3 PROCESSING OF LEAF MATERIAL FOR SOLVENT EXTRACTION

The plucked leaves were rinsed in distilled water to get rid of contaminants adhering to the leaves. The leaves were drawn and air-dried under shade for a week at room temperature. The dried leaves were segregated and pulverized to fine coarse powder in a blender and sieved through 1 mm sieve.

4.11.1.4 PREPARATION OF CRUDE EXTRACTS

Leaf extracts had been organized with the aid of using solvent extraction with Ethyl acetate. a hundred grams of weighed sieved petal powder became extracted with one thousand ml solvent two times with in a single day incubation at room temperature for forty eight hours. The extracts had been filtered using Whatmann filterpaper No. 1. The filtrate became processed in a vacuum evaporator beneath decreased stress to get better the extra solvents for in addition use. The sticky extract received became dried in an oven at 32 ° C and saved in vials at four ° C for in addition analysis..

4.11.2 QUALITATIVE PHYTOCHEMICAL SCREENING OF THE FLORAL EXTRACTS

Qualitative phytochemical screening of the prepared Ethyl acetate extracts were performed as per the protocols stated by Sofowora (1993), Trease (1989), Kokate (1999) to elucidate the constituents present in the leaves of *Ocimum sanctum*.

4.11.2.1 TEST FOR CARBOHYDRATES

5 ml of distilled water was added to individual extracts and dissolved. The vortexed mixture was filtered and the filtrate was used to screen for the presence of Carbohydrates.

FEHLING'S TEST

The filtrate was hydrolysed with dilute HCl acid and neutralised with alkali. It is heated in water bath with the addition of 6-7 drops of Fehlings A & B solution. Appearance of red coloured precipitate indicates the presence of Carbohydrates.

4.11.2 .2 TEST FOR GLYCOSIDES

50 mg of extract was hydrolysed with concentrated HCl for a duration of 2 hours by keeping in a water bath. The solution was filtered and the filtrate was subjected to further tests.

BORNTRAGER'S TEST

3 ml of chloroform was added to 2 ml of filtrate and shaken well. On separation of the two phase, chloroform layer was pipetted out and 10 % of ammonia solution was added in drops. Appearance of pink colour indicates the presence of Glycosides.

4.11.2.3 TEST FOR CARDIAC GLYCOSIDES

KELLER-KILIANI TEST

0.5 ml of extract was treated with 2 ml of glacial acetic acid and 2 drops of 5 % ferric chloride solution. The mixture was underlayered with 1 ml of concentrated H₂SO₄. Brown ring formation at the interface confirms the presence of Cardiac Glycosides.

4.11.2.4 TEST FOR ANTHRAQUINONES

0.5 g of extract was boiled with 10 % of HCl in a water bath for few minutes. the mixture was filtered and cooled. equal volume of CHCl₃ was added in drops. It is further heated gently with the addition of 10 % NH₃. Appearance of rose pink colour indicates the presence of anthraquinones.

4.11.2.5 TEST FOR FLAVONOIDS

ALKALINE REAGENT TEST

0.5 g of extract was treated with few drops of NaOH solution. Appearance of intense yellow colour which disappears on addition of dilute acid confirms the presence of flavonoids.

4.11.2.6 TEST FOR ALKALOIDS

MAYER'S TEST

Extracts were dissolved in dilute HCl individually and filtered . The filtrate was treated with 2-3 drops of Mayer's reagent (Potassium Iodide). Formation of yellowish green or creamy white precipitate indicates the presence of alkaloids.

4.11.2.7 TEST FOR TANNINS

1 ml of extract was treated with 2 ml of 5 % ferric chloride solution. Formation of dark blue or greenish black colour confirms the presence of Tannin.

4.11.2.8 TEST FOR STEROIDS

SALKOWSKI'S TEST

1 ml of extract was treated with 2 ml of Chloroform and 2 ml of Concentrated H_2SO_4 and shaken well. Formation of red colour at the chloroform layer and greenish fluorescent yellow colour at the acid layer indicated the presence of steroid (Terpenoid).

LIEBERMANN BURCHARD TEST

To 2 ml of extract, 1 ml of Liebermann-Burchard reagent was added and shaken well. Formation of blue-green colour indicates the presence of triterpenoids.

4.11.2.9 TEST FOR PHENOLS

1 ml of extract was treated with 2 ml of distilled water followed by the addition of few drops of 10 % ferric chloride solution. The formation of blue or green colour confirms the presence of phenol.

4.11.2.10 TEST FOR AMINO ACID

NINHYDRIN TEST

2 ml of plant extract was treated with 2-3 drops of 0.2% Ninhydrin reagent and heated for 5 minutes in a water bath. Formation of blue colour indicates the presence of protein.

PRELIMINARY ANALYSIS OF LEAVES OF *OCIMUM SANCTUM* HARVESTED FROM CONTROL PLOT

PHYTOCHEMICAL CONSTITUENTS	OBSERVATIONS			
	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract
Carbohydrates	-	-	-	++
Glycosides	+	-	-	-
Cardiac glycoside	-	-	-	+
Anthraquinones	-	-	+	-
Flavoniods	+	-	-	+
Alkaloids	-	-	++	+
Tannins	-	+	+	+
Steroids	-	-	+	+
Phenols	+	+	-	++
Aminoacids	-	-	++	-

+++ Appreciable amount

++ Moderate level

+ Mild level - Absent

TABLE 4. 9

**PRELIMINARY ANALYSIS OF LEAVES OF *OCIMUM SANCTUM* HARVESTED FROM
TEADUST VERMICOMPOST**

PHYTOCHEMICAL CONSTITUENTS	OBSERVATIONS			
	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract
Carbohydrates	-	-	-	+++
Glycosides	+	-	-	-
Cardiac glycoside	-	-	-	++
Anthraquinones	-	-	+	-
Flavoniods	++	-	-	+
Alkaloids	-	-	+++	++
Tannins	-	++	++	+
Steroids	-	-	++	+
Phenols	+	+	-	+++
Aminoacids	-	-	+++	-

+++ Appreciable amount

++ Moderate level

+ Mild level

- Absent

TABLE 4.10

**PRELIMINARY ANALYSIS OF LEAVES OF *OCIMUM SANCTUM* HARVESTED FROM
PRESS MUD VERMICOMPOST**

PHYTOCHEMICAL CONSTITUENTS	OBSERVATIONS			
	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract
Carbohydrates	-	-	-	+++
Glycosides	+++	-	-	-
Cardiac glycoside	-	-	-	+++
Anthraquinones	-	-	++	-
Flavoniods	++	-	-	+
Alkaloids	-	-	+++	++
Tannins	-	++	+++	++
Steroids	-	-	++	++
Phenols	+	+	-	+++
Aminoacids	-	-	+++	-

+++ Appreciable amount

++ Moderate level

+ Mild level

- Absent

TABLE 4.11

**PRELIMINARY ANALYSIS OF LEAVES OF *OCIMUM SANCTUM* HARVESTED
FROM VEGETABLE WASTE VERMICOMPOST**

PHYTOCHEMICAL CONSTITUENTS	OBSERVATIONS			
	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract
Carbohydrates	-	-	-	+++
Glycosides	+++	-	-	-
Cardiac glycoside	-	-	-	+++
Anthraquinones	-	-	++	-
Flavoniods	++	-	-	+
Alkaloids	-	-	+++	++
Tannins	-	++	+++	++
Steroids	-	-	++	++
Phenols	+	+	-	+++
Aminoacids	-	-	+++	-

+++ Appreciable amount

++ Moderate level

+ Mild level

- Absent

TABLE 4.14

**PRELIMINARY ANALYSIS OF LEAVES OF *OCIMUM SANCTUM* HARVESTED
FROM CHEMICAL FERTILIZER**

PHYTOCHEMICAL CONSTITUENTS	OBSERVATIONS			
	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract
Carbohydrates	-	-	-	+
Glycosides	+	-	-	-
Cardiac glycoside	-	-	-	+
Anthraquinones	-	-	+	-
Flavoniods	++	-	-	+
Alkaloids	-	-	+	+
Tannins	-	++	+	+
Steroids	-	-	+	+
Phenols	++	++	-	+
Aminoacids	-	-	+	-

TABLE 4.15

+++ Appreciable amount

++ Moderate level

+ Mild level

- Absent

4.11.3 QUANTITATIVE PHYTOCHEMICAL SCREENING OF LEAVES OF OCIMUM SANCTUM

Quantitative phytochemical screening of flowers was carried out by air drying the leaves of *Ocimum sanctum* for 5 days in shade at 37°C. about 100 grams of dried petals were powdered in a blender, sieved and were subjected to analysis. The tests were performed following the protocols of Harborne (1998).

COMPOSITION	QUANTITY				
	CP	TDVP	VWVP	PMVP	CFP
Protein (grams)	54.33	69.57	71.02	66.87	12.54
Carbohydrates (grams)	9.32	11.69	15.21	13.99	4.69
Fat (grams)	0.01	0.57	0.21	0.87	0.02
Vitamins					
Vitamin A (IU)	14.32	17.51	19.63	24.34	9.48
Folic Acid (mg)	1.23	4.32	10.32	16.57	2.29
Vitamin B ₁ (mg)	0.31	2.35	6.78	5.32	1.87
Vitamin B ₂ (mg)	0.12	0.87	1.24	0.98	0.04
Vitamin B ₆ (mg)	0.36	0.91	0.65	1.07	0.12
Vitamin B ₁₂ Traces	Traces	Traces	Traces	Traces	Traces
Vitamin C (mg)	1.28	3.45	7.24	5.11	0.47
Minerals					
Calcium (mg)	23.07	47.31	49.57	74.39	14.98
Magnesium (mg)	12.7	23.69	41.87	35.78	8.54
Zinc (mg)	0.547	1.987	0.893	1.598	0.486
Iron (mg)	1.36	1.98	2.34	1.57	0.87
Copper (mcg)	0.14	0.51	0.87	0.67	0.54
Sodium (mg)	45.2	58.3	39.2	49.7	12.7
Potassium (mg)	23.8	38.6	32.4	57.3	9.65
Niacin (mg)	3.2	5.9	5.7	9.8	1.8
Phytochemicals					
Steroids (mg)	0.17	0.21	0.14	0.28	0.08
Phenols (mg)	0.73	0.89	1.02	1.27	0.45
Tanins (mg)	1.56	1.98	1.35	1.63	1.19
Flavonoids (mg)	2.67	2.94	3.11	2.88	2.35
Glycosides (mg)	3.24	4.36	3.98	4.11	2.67
Amino Acids (grams)	1.57	4.69	3.21	5.01	0.98
Fatty Acids (grams)	0.54	0.87	1.02	0.98	0.32
Alkaloids (mg)	0.459	0.532	0.721	0.635	0.117

*****Composition as per analysis/100 grams of dried and powdered leaf sample****

TABLE 4.16

4.11.3.1 VOLATILE CONSTITUENTS STUDY OF LEAVES OF OCIMUM SANCTUM

Volatile analysis of the plants grown under the influence of various organic and inorganic amendments was done for the leaf samples.

4.11.3.2 HARVEST OF LEAVES FOR VOLATILE STUDY

Healthy leaves of *Ocimum sanctum* was handpicked in the early hours and was collected in wet cloth bags and carried to laboratory for analysis. It was processed for volatile analysis immediately.

4.11.3.3 PROCESSING OF LEAVES FOR VOLATILE ANALYSIS

Organic solvent extraction protocol was adopted for the extraction of fragrance volatiles (Gochev *et al.* 2008), The leaves were separated and was agitated in a cold percolation chamber with n-Hexane in 1:3 ratio for 24 hours. The extract was distilled at 78° C in rotary evaporator under low pressure to remove the solvent and waxy concrete. The waxy concrete contains essential oils, waxes resins etc which was further extracted twice with ethanol in the ratio of 1:1.5. and the resulting slurry was freezed. The enriched essential oil which is a concentrate was directly used for GC-MS analysis.

4.11.3.4 ANALYSIS OF VOLATILE CONCENTRATE BY GC-MS

The crude concentrate thus obtained was analyzed by GC-MS for the identification of essential compounds. Due to the low temperatures applied in this process, the absolute may be more faithful as the [scent](#) of the fresh rose. This study revealed about the variations in volatile scented compounds in leaves of *Ocimum sanctum* grown under various organic and inorganic amendments.

4.11.3.5 ANALYSIS OF THE VOLATILE CONSTITUENTS

A GC-MS study was carried out at Bureau Veritas laboratories, Chennai, Tamilnadu. GCMS analysis of the volatile extract was performed using GC shimadzu QP2010 model system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with elite I fused silica capillary column (length : 30.0 m, Diameter : 0.25mm, Film thickness : 0.25 composed of 100 % Dimethyl poly siloxane). An electron ionization energy system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51 ml/min and an injection volume of 2iL was employed with a split ratio of 20. Injector temperature and ion-source temperature were maintained at 200°C; the oven temperature was programmed from 70

°C (isothermal for 2 minutes), with an increase of 300°C for 10 minutes. mass spectra were taken at 70eV at a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 minutes. The relative percentage amount of each component was calculated by compaing its average peak area to the total peak areas. Mass spectra patterns were prepared by using GC-MS solution software version of 2.53.

4.11.3.6 IDENTIFICATION AND INTERPRETATION OF COMPOUNDS

Interpretation of spectral patterns of GC-MS was conducted by accessing the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having standard patterns. The spectrum of unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The Name, Molecular weight, Molecular formula and structure of the component of the test material were correlated and the results were recorded.

S.No	Components	Retention Time/Peak Percentage				
		Control plot	TDVP	PMVP	VWVP	CFP
1	Benzene acetaldehyde	3.01 0.27	6.01 0.15	12.99 0.69	13.22 0.14	2.01 0.87
2	Phenyl ethyl alcohol	4.42 0.86	7.48 1.02	16.07 32.93	15.95 28.50	3.39 2.74
3	Citronellol	5.01 2.89	9.01 2.04	17.36 0.71	12.54 0.91	2.01 2.49
4	Geraniol	2.38 0.20	4.37 0.31	9.37 0.59	6.38 0.37	5.37 0.49
5	2- Phenyl ethyl ester acetic acid	5.44 2.98	9.44 5.43	20.82 2.17	14.02 2.29	5.07 3.57
7	Henicosane	11.37 4.74	19.19 4.01	47.43 5.20	47.41 2.00	15.37 4.77
8	Eicosane	8.11 0.45	20.97 4.42	49.34 0.43	45.26 0.21	18.24 0.34
9	Behenic alcohol	22.56 1.43	24.10 3.97	53.70 1.04	53.76 5.93	21.74 0.09
10	Nonadecane	-	17.26 2.61	42.85 2.87	29.67 1.12	2.67 2.31
12	Eugenol	-	10.91 0.42	24.15 0.28	-	8.73 0.38
13	Geranyl isobutyrate	24.46 0.74	25.87 1.33	55.81 1.03	-	18.54 2.57
14	Geranyl	6.89	36.98	54.82	37.63	10.98

	acetate	0.43	1.09	1.46	2.37	0.44
15	Mesitylene	2.69 0.10	5.09 0.09	11.28 0.09	8.21 0.21	3.87 1.24
16	Bis (2 - ethyl hexyl) phthalate	-	22.86 0.41	54.95 1.01	32.69 0.37	22.88 0.59
17	Alpha amyrin	14.65 1.37	-	30.25 3.69	21.87 2.93	19.87 2.54
18	Beta amyrin	12.69 1.87	-	-	-	6.35 2.57
19	Longipinane	15.32 0.98	23.68 1.57	26.87 0.36	34.87 2.68	19.68 0.58
20	Styrene	3.31 0.13	-	-	-	3.31 0.25
21	Azulene	-	21.45 0.18	-	18.26 0.14	8.45 0.13
22	E – 7 Octadecene	12.50 0.36	18.00 0.15	-	-	11.57 0.98
23	Alpha Guaiene	9.65 0.11	11.98 0.16	-	26.69 0.03	11.98 0.15
24	8 – Heptadecene	9.24 0.27	14.87 0.18	42.11 2.87	29.64 1.47	15.54 2.57
25	Vitamin – E	0.65 1.29	1.59 0.39	2.54 1.55	1.99 0.73	0.33 1.89

TABLE 4.17

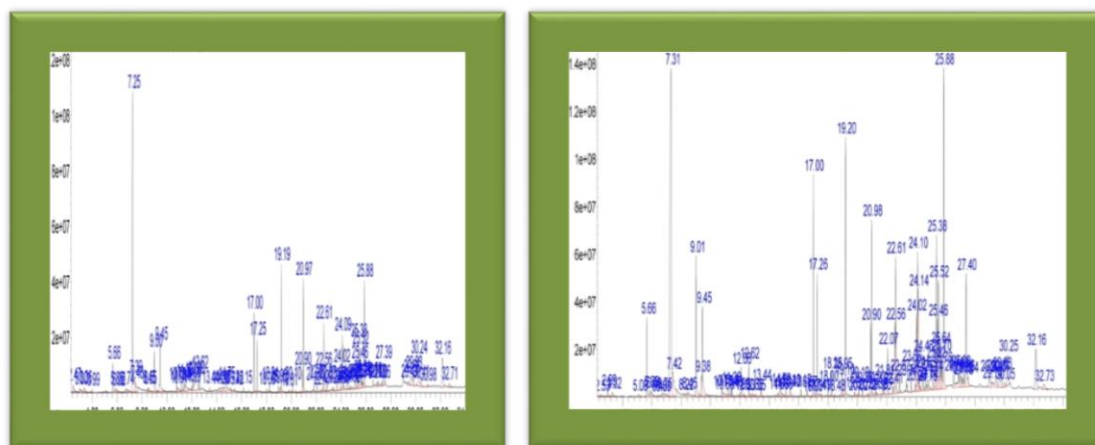


FIGURE 4.14

CHAPTER 5 RESULTS AND DISCUSSIONS

5.1 ORGANIC WASTE

Organic waste is an inseparable element of human existence and activity. They include agricultural waste, animal and human waste, catering and household food waste etc. Role of earthworms in the breakdown of organic debris on soil surface and soil turn over process was first highlighted by Darwin (1881). Since then it has taken almost a century to appreciate their important contribution in curbing organic pollution and providing topsoil to impoverished lands. Although this realization has awakened the global population to give a serious thought to utilize them for the benefit of mankind, by the turn of this century.

Environmental degradation is a major threat confronting the world, and the rampant use of chemical fertilizers in agriculture contributes largely to the deterioration of the environment through depletion of fossil fuels, generation of carbon dioxide (CO₂) and contamination of water resources. It leads to loss of soil fertility due to imbalanced use of fertilizers that has adversely impacted agricultural productivity and causes soil degradation. Now there is a growing realization that the adoption of ecological and sustainable farming practices can only reverse the declining trend in the global productivity and environment protection Wani and Lee (2002).

5.2 VERMICOMPOST

Vermicomposting is a process of composting, in which certain species of earthworms are used to enhance the process of waste conversion and produce a better end product. The process is faster than composting because the material passes through the earthworm gut where a significant but not yet fully understood transformation takes place, resulting in earthworm castings (worm manure) that are rich in microbial activity and plant growth regulators and also fortified with pest repellent attributes. Earthworms, through a type of biological alchemy, are capable of transforming garbage into 'gold' (Vermi C, 2001).

Vermicompost is a nutritive 'organic fertilizer' rich in NPK, micronutrients, beneficial soil microbes like nitrogen-fixing bacteria and mycorrhizal fungi and are scientifically proving as a miracle growth promoters and protectors (Sinha *et al.*, 2009).

In the present study the different vermicomposts were used to enhance the phytochemical and volatile constituents of leaves of *Ocimum sanctum*. In this contrast, the physico-chemical characteristics of the prepared vermicompost are very important in determining the quality of the prepared vermicompost. The present study provides data suggesting beneficial role of the earthworm *Perionyx excavatus*, in composting and stabilizing nutrients in the vermicompost prepared from different organic wastes.

5.2.1 PHYSICO-CHEMICAL PROPERTIES OF VERMICOMPOST

The study made with the following organic wastes and the selected worm species have visualized some unopened secrets of vermicomposting. The locally collected worm species have shown tremendous potentiality in converting organic wastes into valuable nutrient rich organic manure with wide range of physico-chemical characteristics among different vermicomposts prepared. The present study was similar to the findings of John Paul *et al.* (2011).

5.2.2 PHYSICO-CHEMICAL PROPERTIES OF CULTIVATION BED AMENDED WITH VARIOUS AMENDMENTS

The study with the supplements of various organic and inorganic amendments for the cultivation of *Ocimum sanctum* showed a wide range of nutritional parameter variations during the overall period of cultivation in the study plot. The nutrient profile of chemical fertilizer amended plot was very high beyond the saturation limits which will deteriorate the physical structure, respiratory quotient, electrical conductivity, water holding capacity and the beneficial microbial flora of the soil there by stressing plants grown under such conditions.

Soil is generally considered fertile when it has good physical structure, balanced nutrients and sufficient biotic activity (Mader *et al.*, 2002). Vermicompost the 'soil conditioner' are materials characterized by high porosity, aeration, drainage, water holding capacity and high content of microbial activity (Atiyeh *et al.*, 2000) which is made up of primarily C, H and O, and holds nutrients such as NO₃, PO₄, Ca, K, Mg, S and micronutrients in plant available forms exhibiting significant effects on the growth and yield of plants as like the inorganic fertilizer but in an eco-friendly manner.

Plants obtain nutrients from soil through soil reserves, crop residues, vermicomposts, synthetic and other nutritive amendments. These nutrients are essential for plant growth and development and their deficiency may result in poor plant growth (Welch, 2002). Therefore for cultivating plants like *Ocimum sanctum*, a continuous supply of both macronutrients like N, P, K, Ca, Mg, S and micronutrients like Fe, Cu, Zn, Mn, Mo, B is crucial for a successful harvest without compromising nature. The prepared vermicompost from TDVC, PMVC, VWVC showed a gradual increase of soil physico-chemical properties during the cultivation period.

5.2.3 NPK CONTENT

The level of nitrogen, phosphorous, potassium was found to be gradually increasing in the vermicompost applied plots. Nitrogen plays a significant role in photosynthesis, cell division and differentiation, growth and somatic embryogenesis, chlorophyll content, rubisco activity, electron transport rate, photosynthetic rate, anthocyanin production and an important component of proteins required for the metabolic reactions that take place during plant growth (Jain *et al.*, 1999). Phosphorous also plays a role in increasing water-use efficiency (WUE), a critical factor for plant productivity in hot climates (Wittenmayer and Merbach, 2005), improves leaf expansion, axillary bud growth and shoot canopy, improved photosynthetic surface area and carbohydrate utilization (Ahloowalia *et al.*, 2004). Potassium plays a vital role in many physiological processes essential for plant growth, including the maintenance of plant water balance and protein synthesis.

Even though the physical parameters like soil bulk density, aggregate formation, water holding capacity, anion exchange capacity was not studied in the present work, studies have confirmed the positive role of vermicompost in increasing these parameters (Weber *et al.*, 2007) which cannot be obtained with chemical fertilizers. The soil organic carbon was found to be high among vermicompost treated plots which correlate the findings of Nardi *et al.* (2004) and Weber *et al.* (2007).

5.2.4 MAGNESIUM AND CALCIUM CONTENT

Magnesium and calcium content in soil was in appreciable amount in the vermicompost treated plots. Magnesium is involved in numerous physiological and biochemical processes activating more enzymes than any other mineral nutrient, thus, making a significant contribution to plant growth and development (Epstein and Bloom, 2004). Calcium plays an important role in a wide range of physiological and biochemical processes in plants including the adjustments of ethylene responses in plants (Zhang *et al.*, 2002).

5.2.5 MICRONUTRIENTS

The level of micronutrients such as Fe, Cu, Zn, Mn and B were in considerable amount in the vermi plots. The micronutrient assists in the formation of chlorophyll, cell division and growth, carbohydrate metabolism, as well as in the maintenance of plant's enzyme system (Follet *et al.*, 1981). Therefore, the type of organic or inorganic fertilizers used in agriculture with different quantities of macro and micronutrient will have a significant effect on the nutrient value of the consumable plants.

Studies of Kumari and Ushakumari (2002) reported that treatment with enriched vermicompost was superior to other treatments for the uptake of N, P, K, Ca and Mg in their study with cowpea (*Vigna unguiculata* L. Walp). Similar such findings were observed by Sainz *et al.* (1998) who have reported that addition of vermicompost to soil resulted in an increased mineral contents in the substrate and higher concentrations of P, Ca, Mg, Cu, Zn and Mn in shoot tissues of red clover and cucumber. This proves nutrients in vermicompost are easily absorbed by plants which is evident from the accumulation of minerals in the plant parts. This outcome is similar to the present findings.

Studies have confirmed that plants fertigated with vermicompost have shown greater ability to assimilate essential macro and micro nutrients from the soil, and resulted in an improved root development (Arancon *et al.*, 2006). Hence it is possible to cultivate plants for well developed root system which in turn can absorb nutrients as per the requirement.

Nutrient richness of plots supplemented with TDVC, PMVC and VWVC containing rich micro and macro nutrients can trigger photosynthesis by the formation of

chlorophyll which is required for light harvesting and subsequent conversion into chemical energy via photo assimilation (Tanaka *et al.*, 1998). Hence *Ocimum sanctum* cultivated under such conditions will have increased rate of photosynthesis which can lead to an increased growth facilitating profitable yield.

Hence in the study, plants treated with chemical fertilizer will have reduced rate of photosynthesis due to high accumulation of micro and macro nutrients which will not produce a profitable yield in *Ocimum santum*. Berova and Karanatsidis (2009) have reported for an increased level of photosynthetic pigments and leaf gas exchange in red chilly (*Capsicum annum* L.) amended with vermicompost. In a separate study, Golchin *et al.* (2006) reported that leaf area index (LAI) and chlorophyll content of the leaves of pistachio (*Pistacia vera*) seedlings, as well as the photosynthesis rate were better in vermicompost treatments relative to the treatments without vermicompost. Similarly, apart from the increased leaf area and photosynthetic pigments in a red pepper (*Capsicum annum*), the speed of the net photosynthesis was 33% higher in treatments with vermicompost compared with the control plants (Berova and Karanastidis, 2008).

Vermicompost with its rich content of micro and macro nutrients, organic matter content, organic carbon and optimum electrical conductivity levels can be the only option of cultivating plants for an increased yield and also for maintaining the soil fertility. For example, an organic source of plant nutrients contains a higher percentage of nutrients necessary for plant growth in readily available forms (Nagavallemma *et al.*, 2004).

As a result, vermicompost has a potential for improving plant growth and dry matter yield when added to the soil (Zaller, 2007). Studies have shown that vermicompost plays a major role in improving growth and yield of different field crops, including vegetables, flowers and fruit crops. Similar findings with the application of vermicompost were recorded for higher germination, growth yield of mung bean (*Vigna radiata*) compared with the control were observed by (Nagavallemma *et al.*, 2004). In a study involving a wide range of vegetable and ornamental seedlings, result showed earlier and better germination in a vermicompost compared with control (Gutierrez-Miceli *et al.*, 2007).

Plant growth and development is affected by the rates of the vermicompost applied to the soil which are in turn is affected by agro-climatic conditions of the growing

environment (Atiyeh *et al.*, 2000). When used at lower substitution rates, vermicomposts can increase growth, flowering and yields of vegetable and ornamental crops.

5.3 ENZYME ACTIVITY IN THE CULTIVATING FIELD SOIL

In addition to the changes exerted on the physico-chemical properties of soil, worm mediated composted materials have a clear impact on soil biological properties, such as increase in microbial biomass and activity (Knapp *et al.*, 2010), as well as changes in the activity of soil enzymes (Garcia-Gil *et al.*, 2000) and in the structure of the soil microbial community (Ros *et al.*, 2006).

Enzyme activity of the study plot showed a wide range of deviations among the different vermicompost treatments used in the study. Since these enzymes are considered to be excellent indices of overall microbial metabolic activity (Nannipieri *et al.*, 1990), increased enzyme activity in the study plot indicates increased microbial populations involved in metabolic activity. Vermicompost treated plots were found to show an increased level of enzymatic activity comparing to the chemical fertilizer supplemented plots. IAA, dehydrogenase, urease, acid phosphatase, alkaline phosphatases and catalase activity was found to be higher in the plot amended with vermicompost, while chemical fertilizer amended plot was found to show an overall decline trend of enzyme activity of the cultivation bed during the study.

The present study with the application of 6t/ha of vermicompost were found to significantly increase in the level of enzyme activity in the study plot. Studies by (Tiwari *et al.*, 1989) have observed similar results with the annual application of adequate amount of vermicompost and reported for a significant increase in soil enzyme activities such as urease, phosphomonoesterase, phosphodiesterase and arylsulphatase in garden soils. Similar results of increased glucosidase, protease and alkaline phosphomonoesterase enzyme activity rates were observed in soil plots amended with vermicompost and manure than in those treated with inorganic fertilizer (Cristina Lazcano and Jorge Dominguez, 2011). There have been many similar reports that organic fertilizers can increase soil microbiological activity (Marinari *et al.*, 2000). Masciandaro *et al.* (1997) reported an increase in soil enzyme activity after

vermicompost applications to soils at rate of 90 t/ha. Hence, vermicompost can be considered as the best organic manure for increased yield and in maintaining soil fertility than chemical fertilizer since enzyme activity was less evident in the chemical fertilizer amended plots. Hence application of vermicompost can induce soil fertility, nutrient cycling and reclamation promoting plant growth, while chemical fertilizer is said to destruct the precious cultivable lands deteriorating the entire beneficial microbial consortium making the land unfit for crop cultivation. Two of the major contributions of vermicomposts to the field soils were the increased microbial populations and activities which are key factors in rates of soil nutrient cycling, production of plant growth-influencing materials, and the build-up of plant resistance or tolerance to crop disease and nematode attacks. This could have been achieved by the rich microbial populations in the vermicomposts that inoculated and spread through the soil.

5.3.1 MICROBIAL LOAD IN THE CULTIVATING FIELD SOIL

The quality and value of agricultural organic soil amendments are often measured in terms of their contributions to nutrient supplies and soil fertility. However, they can also have significant effects on the microbiological and chemical properties of the soil promoting organic matter decomposition, which are indirectly responsible for supporting crop growth (Nannipieri *et al.*, 1990).

The supplement vermicompost has highly influenced in an increase of microbial colonies in the cultivation bed of *Ocimum sanctum*. Both the bacterial and the fungal loads were found to be significantly increased among plots treated with vermicompost. Research works of Pascual *et al.* (1999) reported significant increase in the microbial biomass N following the addition of food waste vermicompost in field soils.

The least content of microbial loads were observed in the plot treated with chemical fertilizer. This again confirms the destructive role of chemical fertilizer in altering the microbial consortium of the cultivation soil. The reason for increased microbial load in the control plot might be due to the presence of decomposed organic matter in the cultivation soil that might have attracted rich microbial colonies to feed upon them. Increase in microbial populations and activities are key factors that influence the rate of nutrient cycling, production of plant growth-regulating materials,

and the build-up of plant resistance or tolerance to crop pathogen and nematode attacks (Arancon *et al.*, 2006). However, information regarding the impacts of vermicompost on soil microbial properties is still limited. A single application of vermicompost to a strawberry crop has been shown to produce a significantly higher increase in soil microbial biomass than application of an inorganic fertilizer, independently of the dose used (Arancon *et al.*, 2006). As well as increasing microbial biomass, vermicompost increases microbial activity (Ferrerias *et al.*, 2006) promotes the establishment of a specific microbial community in the rhizosphere different from that of plants supplemented with mineral fertilizers or other type of organic fertilizers such as farm yard manure (Aira *et al.*, 2010). Studies have confirmed with highly significant increase in soil enzyme activity by vermicompost addition as compared to equivalent rates of mineral fertilizers (Saha *et al.*, 2008). From the study it is concluded that use of this type of organic fertilizer therefore has great potential in the growth of *Ocimum sanctum*.

Phytochemicals are naturally occurring chemical compounds found in plants. Phytochemicals work as a defense mechanism and are known to protect plant from various pathogens. Recent researches have proved that such phytochemicals can protect human beings from various diseases in a broad range of spectrum. Plants are rich in wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, glycosides, saponins and flavonoids. These metabolites are found in varying quantities in various plant species and show considerable variation according to their geographical distribution. These phytochemicals are known to possess antibacterial, antifungal, antioxidant, anti-inflammatory and anticancer properties

5.4 QUALITATIVE PHYTOCHEMICAL PROFILE OF LEAVES OF *OCIMUM SANCTUM*

Preliminary phytochemical screening of the leaves of *Ocimum sanctum* from the 5 plots of study (CP, TDVP, PMVC, VWVP and CFP) were tested positive for the presence of different secondary metabolites in different qualitative variance. The positively tested phytochemicals includes carbohydrates, glycosides, cardiac glycosides, anthraquinones, flavonoids, alkaloids, tannins, steroids, phenols and aminoacids in their respective solvents. Levels of secondary metabolites were higher in the samples from the vermicompost amended plot (TDVP, PMVP and VWVP) than that grown in the

inorganic (CFP) and control plot (CP).

These phytochemicals are known to play an important role in the branch of medicine. Flavonoids are known to carry out antioxidant, protective effects and inhibit the initiation, promotion and progression of tumor cells (Okwu, 2004) and also take part in the modulation of estrogen levels in humans (Okwu and Omodamiro, 2005).

Phenolics are known to suppress the growth and development of pathogens.

5.5 QUANTITATIVE PHYTOCHEMICAL PROFILE OF LEAVES OF *OCIMUM SANCTUM*

Quantitative phytochemical analysis of dried powdered leaves of *Ocimum sanctum* has revealed significant degree of variations among the samples tested from 5 plots (CP, TDVP, PMVP, VWVP and CFP). The samples from the organic plot has recorded higher quantification of parameters like carbohydrates, glycosides, cardiac glycosides, anthraquinones, flavonoids, alkaloids, tannins, steroids, phenols and aminoacids while comparing to the samples from the inorganic (CFP) and the control plot (CP). The quantitative biochemical analytical results in the present study indicate high level of metabolic rates of plants grown under the influence of vermicompost. The enhanced rate of variation of different phytochemical constituents in the leaves of *Ocimum sanctum* clearly indicates the high rate of metabolism and growth of plants amended with vermicomposts.

The rate of biosynthesis of these secondary metabolites is directly influenced by the fertilizer application (Grevsen *et al.*, 2008). In the present study an increase in the phytochemical content in the leaves of *Ocimum sanctum* is observed in the petal samples from the plot treated with vermicompost while a lower level in the inorganic and control plots. Increase in the levels of phenols have been reported in strawberries and marionberries treated with vermicomposts (Danny *et al.*, 2003). Several studies have also indicated the same results on medicinal plants (Darzi *et al.*, 2007). The present findings is well supported by the findings of Mitchell (2007) who have reported through a decade of research that levels of flavonoids, quercetin and kaempferol in organic tomatoes were 79 % and 97 % higher than those recorded in the chemically grown tomatoes respectively.

Also the levels of flavonoids increased over time in the tomato samples cultivated organically. Previous studies also indicate that organic foods are rich in organic acids and poly-phenolic compounds many of which is essential antioxidants necessary for health maintenance (Winter and Davis, 2006). Potatoes grown under the influence of organic fertilizer are found to contain rich proportions of ascorbic acids comparing to those grown chemically. The increase in the content of vitamin C in the leaves of *Ocimum sanctum* cultivated under organic amendment is well correlated with the findings of Shankar and Sumathi (2008) who have reported high content of vitamin C in spinach, pears, potato, pepper, tomato, celery, turnip, apple, cabbage, carrots and beetroots grown organically. Such results were well acknowledged by Ismail (2003) who have observed an increased content of β - carotene, vitamin C and roboflavin contents in organically grown cabbage. Further, an increase in proteins by 4-6% in spinach, 33-40% in savoy, 15-24% in lettuce, 24-37% in celeriac, 21-25% in carrots and trace level in potatoes was also reported. The present study also showed similar trend of increase in secondary metabolites.

The increased level of steroids was observed in the leaf extracts of *Ocimum sanctum* from the plots supplemented with vermicompost in the present study. However decreased levels of steroids in plants were reported by Aliero *et al.* (2006) in *Solanum pseudocapsicum* and Osuagwu (2008) in *Ocimum gratissimum* which was grown under the influence of organic fertilizer and vermicompost was not used in their study. It has been shown earlier that the addition of organic matter improves soil properties such as aggregation, water holding capacity, hydraulic conductivity, bulk density, the degree of compaction, fertility and resistance to water and soil erosion. These soil factors positively affect the growth and development of plants and also the metabolites. The positive effects of organic nutrient application on secondary metabolites content are in agreement with the previous reports on other medicinal plants, such as mint (Ram *et al.*, 2006), basil (Sifola and Barbieri, 2006), fennel (Kapoor *et al.*, 2004) and artemisia (Jha *et al.*, 2011).

This present study on comparative effect of organic and inorganic fertilizers on phytochemical constituents revealed that vermicompost imparted higher effects in phytochemical constituents when compared to the inorganic fertilizer. The results of the present study is also in agreement with the previous studies of Funda *et al.* (2011) and

Makinde *et al.* (2010) who have reported an increase of some proximate and mineral content with the application of organic fertilizers.

The reason of biochemical richness in the present study could be due to the fact that vermicomposts conditions the soil with rich population of beneficial soil microbes and essential nutrients important for plant growth thus reinforcing its natural fertility. Whereas, the chemical fertilizers destroy the beneficial microbes and impair the natural fertility of soil while affecting soil pH and porosity and hence affects the profile of phytochemical constituents. Plants cultivated under such conditions are nutrient deficient. The results are encouraging but precise antimicrobial assessment is necessary before commencing cultivation of plant with rich therapeutic and pharmacological properties.

CHAPTER 6 SUMMARY AND CONCLUSIONS

There is always a global importance for organic farming through recycling of organic wastes for sustainable agriculture as well as for an eco-friendly and pollution-free environment and ecosystem in which we live. Use of earthworm species, *Perionyx excavatus* in vermicomposting of organic wastes for the production of agronomical important vermicompost is very important for commercialization because loss of nitrogen and toxic gases from agricultural wastes and cow dung is minimized when vermicompost is the source of organic matter. The present study was framed to assess the potential of locally available earthworm *Perionyx excavatus* in composting of different types of organic wastes (press mud, tea dust and vegetable waste) and to analyze the nutrient quality, enzyme activity and microbial load in soil following vermicompost application. Further, studies were conducted to compare the effect of these vermicomposts and commercial fertilizer in the qualitative, quantitative and volatile constituents of leaves of *Ocimum sanctum* in field condition.

The locally collected organic wastes like tea dust, press mud and vegetable waste were composted using the earthworm *Perionyx excavatus* into nutrient organic manure. The physico-chemical properties of the field soil were tested at the start of cultivation and during the course of cultivation. Enzyme activity like IAA, dehydrogenase, urease, acid phosphatase, alkaline phosphatase and catalase activity

differed among different plots amended with different vermicompost.

Cultivation soil was subjected to microbial analysis. Total bacterial and fungal populations were maximum in PMVC followed by TDVC, VWVC, CP and CFP. Experiments were conducted to analyze the component of phytochemicals present in a fixed quantity of dried leaf powder obtained from the CP, TDVC, PMVC, VWVP and the CFP. Tests were conducted for carbohydrates, glycosides, cardiac glycosides, anthraquinones, flavonoids, alkaloids, tannins, steroids, phenols and amino acids.

Protein, carbohydrates and fat were present in appreciable quantity in the samples of the PMVP comparing to the other samples of *Ocimum sanctum*. Similarly vitamins A, B1, B2, B6, C and folic acid were present in all the tested samples while their quantity was fairly more in the sample from the PMVP. The experiments to identify minerals like calcium, magnesium, zinc, copper, potassium, nitrogen, sodium, iron, potassium and niacin in the samples have confirmed their presence in all the samples tested with varying ranges of quantity. The quantity was less in samples from the CFP and CP.

Volatile Analysis of the leaves of *Ocimum sanctum* from the different treatment plots had identified about 63 volatiles. Of them, only 25 important volatiles were accounted. In these experiments we could observe that most of these volatiles are commonly found in all the samples in minute quantities. Those volatiles that are mostly responsible for the sharp fragrance are phenyl ethyl alcohol, citronellol, geraniol, geranyl acetate, geranyl isobutyrate, hencicosane, eicosane, 2- phenyl ethyl ester acetic acid, nonadecane, etc. These experiments proved that these volatiles were predominantly present at their highest level in the leaf samples from vermicompost treated plots. Highest quantum of citronellol was recorded in the TPVP. Benzene acetaldehyde, Phenyl ethyl alcohol, Geraniol, Henicosane, Nonadecane, Geranyl acetate, Bis (2 ethyl hexyl phthalate) Alpha amyrin, 8 Heptadecene was found to be higher in PMVP. 2 - phenyl ester acetic acid, Eicosane, Azulene, Alpha Guaiene was recorded high in samples from the TDVP. Behenic alcohol and Longipinane was found to be higher in samples from the VWVP.

Earthworms are truly justifying the beliefs and fulfilling the dreams of Sir Charles Darwin who called earthworms as 'unheralded soldiers of mankind and friends of

farmers'. The production of degradable organic waste and its safe disposal becomes the current global problem. Meanwhile the rejuvenation of degraded soils by protecting topsoil and sustainability of productive soils is a major concern at the international level. They serve as "nature's plowman", which is the most precious material to fulfill the nutritional needs of crops. The utilization of vermicompost results in several benefits to farmers, industries, environment and overall national economy.

From the present findings, it can be concluded that the investigation provides a sound basis to declare vermicomposting as a suitable technology for conversion of different types of organic wastes into value added material. Analysis of vermicompost obtained from different organic substrates like tea dust, press mud, and vegetable wastes clearly indicates its utility as soil conditioner and good source of plant nutrients. Vermicomposts applied at very low rates of 6 tons/ha can significantly increased the phytochemical and volatile constituents of leaves of medicinally important *Ocimum sanctum*.

The study visualizes that plants grown under vermicompost is rich in phytochemical constituents which in turn increased their medicinal properties. While, chemical fertilizers decreased their content. Further, the stimulatory effect of vermicomposts in the field enhanced the quality of soils by increasing microbial biomass, which are key components in nutrients cycling thereby preserving the motherly nature.

Novelty of the present work is to authenticate, the fact that creating moderate stress with respect to nutrient management status of soil and sustained availability of the nutrient through integrated nutrient management enhanced the essential oil content and quality of the volatiles in the leaves of *Ocimum sanctum*.

This practice will give boost for commercial crop cultivation and thus we recommend that farmers should be educated about the importance of vermicomposting. This will reduce the additional burden of purchasing costlier synthetic fertilizers that in turn will decrease the pollution load on our environment and to save our precious cultivable lands.

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