KUVEMPU UNIVERSITY

DEVELOPMENT OF A MICROBIAL CONSORTIUM FOR COMPOST PRODUCTION FROM HORTICULTURAL WASTES

Thesis Submitted to Kuvempu University

For the Award of the Degree of

DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY

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DECLARATION

I **Asha. K,** hereby declare that the thesis entitled "**Development of a Microbial Consortium for Compost Production from Horticultural Wastes",** which is submitted herewith for the award of degree of **"Doctor of Philosophy in Microbiology"** to Kuvempu University is the result of the research work carried out by me under the guidance of **Dr. G. Selvakumar**, Principal Scientist (Agrl. Microbiology), Division of Soil Science and Agrl. Chemistry, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Hesaraghatta Lake Post, Bengaluru-560089 and under the Co-guidance of **Dr. B. Thippeswamy**, Assistant Professor, Department of Post Graduate Studies and Research in Microbiology, Kuvempu University, Jnanasahyadri, Shankaraghatta, Shivamogga – 577 451, Karnataka, during the period 2012 to 2017.

I, further declare that, this thesis or any part thereof has not been submitted elsewhere for any degree or diploma in any other University**.**

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CERTIFICATE

This is to certify that the thesis entitled **"Development of a Microbial Consortium for Compost Production from Horticultural Wastes"**, submitted for the award of the degree of **"Doctor of Philosophy" in Microbiology**, by **Asha. K,** is the result of a bonafide research work carried out by her under my guidance during the period from 2012-2017, in the Division of Soil Science and Agricultural Chemistry, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru-560089, Karnataka, India.

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ASHA. K

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LIST OF ABBREVIATIONS

GDP	Gross Domestic Product
Tg	Teragram
O_2	Oxygen
CO_2	Carbon Dioxide
H_2O	Water
NH_4	Ammonium
NO_3	Nitrate
$NaNO_3$	Sodium nitrate
KCl	Potassium chloride
$MgSO_4$	Magnesium sulphate
K_2HPO_4	Dipotassium hydrogen phosphate
$FeSO_4$	Ferrous sulphate
µg	Microgram
g	Gram
mg	Milligram
Kg	Kilogram
g/Kg	Gram/kilogram
IU	International Units
U	Units
Nm	Nanometer
mm	Millimeter
M	Molar
N	Normality
ml	Milliliter
µmol	Micro mole
µS	Micro Siemens
w/v	Weight/volume
h	Hour
min	Minutes
AR	Analytical reagent

DNS	Dinitrosalicylic acid
FPU	Filter Paper Units
FPA	Filter Paper Assay
FAS	Ferrous Ammonium Sulphate
H_2SO_4	Sulphuric acid
NaOH	Sodium hydroxide
CMC	Carboxymethyl cellulose
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
RBBR	Remazol Brilliant Blue R
PDA	Potato Dextrose Agar
SDA	Sabouraud Dextrose Agar
EC	Electrical Conductivity
KI	Potassium iodide
ITS	Internal Transcribed Spacer
DNA	Deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
BLASTn	Basic Local Alignment Search Tool
FYM	Farm Yard Manure
HI	Humification Index
NCBI	National Center for Biotechnology Information
MSA	Mannitol Salt Agar
TAMEA	Tannic Acid Malt Extract Agar
NA	Nutrient Agar
XBM	Xylan Basal Medium
LME	Lignin-Modifying Enzymes
C: N ratio	Carbon-to-Nitrogen ratio
TOM	Total Organic Matter
TOC	Total Organic Carbon
WSC	Water Soluble Carbon
WSN	Water Soluble Nitrogen
FC	Fulvic like Carbon

HC	Humic like Carbon
C_{ext}	Alkali extractable carbon
GI	Germination Index
TV	Titre value
DHA	Dehydrogenase Activity
TPF	Triphenylformazan
HPLC	High-Performance Liquid Chromatography
rpm	Rotations per minute

ABSTRACT

Compost production from horticultural crop residues is a viable and sustainable method of bioconversion in order to overcome the scarcity of good quality organic manures for crop production. Traditionally cow dung has been used as a compost starter to prepare composts, but in recent times the scarcity of cow dung especially in the peri-urban areas and its bulkiness, have led to the use of alternatives such as microbial bioconversion starters for the composting of horticultural wastes. The present study was therefore conducted with aim of developing a microbial starter consortium containing potential bioconversion agents for the bioconversion of different groups of horticultural crop residues *viz,* woody residues (chilli stalks, grape pruning's), succulent residues (*Dolichos* stover), semi-succulent residues (brinjal stalks) and litter (mango leaf litter). Twenty five bacterial and seventy fungal isolates covering the entire bioconversion spectrum *viz.*, cellulolytic bacteria, cellulolytic fungi, xylanolytic fungi, tannin degrading fungi, pectinolytic fungi and lignolytic fungi were isolated from samples collected from compost pits, farmyard manure, fruit and vegetable dumping sites around the periurban regions of Bengaluru. Based on preliminary screening assays, six bacterial isolates and four fungal isolates were selected for their cellulolytic activities, four fungal species for their xylanolytic activities, three fungal species for their tannin degrading abilities, three fungal species for their pectinolytic activities and ten fungal species for their lignin degrading abilities. The individual enzymatic activities of these isolates were quantified by using standard protocols. *In vitro* composting studies using chilli stalks as substrate was used to determine the humification potential of the elite isolates. Based on the compatibility amongst the isolates as determined by the dual culture technique, three microbial starter consortia were formulated and evaluated for the bioconversion of horticultural residues under *in vitro* conditions. It was observed that mango leaf litter and

Dolichos stover were composted within ninety days while the lignolytic substrates such as chilli stalks, grape pruning's and brinjal stalks required one hundred and fifty days for complete composting. The composts prepared using different consortia were evaluated for their maturity and stability parameters per standard protocols. The Consortium-1 comprising (*Aspergillus* sp. TMLF-1, *Aspergillus oryzae* CP-2, *Trichoderma hamatum* FLF-13 and *Aspergillus sp.* TL-8) produced the best quality compost from mango leaf litter (C:N ratio of 15.52, humification index of 4.1, humic like carbon/Fulvic like carbon ratio of 3.3, radish seed germination index of 371.58) and grape pruning's (C:N ratio of 14.4, humification index of 5.2, humic like carbon/ Fulvic like carbon ration of 3.4, radish seed germination index of 431.51). The Consortium-2 comprising (*Bacillus endophyticus* FLCB-11, *Aspergillus sp*. CF-11, *Xylaria* sp. TF-4 and *Penicillium chrysogenum* VCLF-1) produced the best quality compost from chilli stalks (C:N ratio of 16.13, humification index of 6.25, humic like carbon/fulvic like carbon ratio of 6.09, radish seed germination index of 308.2), brinjal stalks (C:N ratio of 14.40, humification index of 4.5, humic like carbon/fulvic like carbon ratio of 4.63, radish seed germination index of 308.21) and *Dolichos* stover (C: N ratio of 14.82, humification index of 4.2, humic like carbon/fulvic like carbon ratio of 3.13, radish seed germination index of 224). When the prepared composts were evaluated under polyhouse conditions on tomato hybrid UNIK-38, it was observed that the mango leaf litter, brinjal stalks and grape pruning's composts prepared using the Consortium-1, significantly improved the vegetative and harvest parameters of tomato, while the *Dolichos* stover and chilli stalks composts prepared using Consortium-2 were superior in improving the vegetative and harvest parameters of tomato. Based on the overall performance of the starter consortia it is concluded that Consortium–2 comprising (*Bacillus endophyticus* FLCB-11, *Aspergillus sp*. CF-11, *Xylaria* sp. TF-4 and *Penicillium chrysogenum* VCLF-1) is

more suitable for the bioconversion of a wide range of horticultural crop residues, while the Consortium-1 comprising comprising (*Aspergillus* sp. TMLF-1, *Aspergillus oryzae* CP-2, *Trichoderma hamatum* FLF-13 and *Aspergillus sp.* TL-8) is more suited for bioconversion of mango leaf litter and grape pruning's.

CHAPTER-1

Introduction

INTRODUCTION

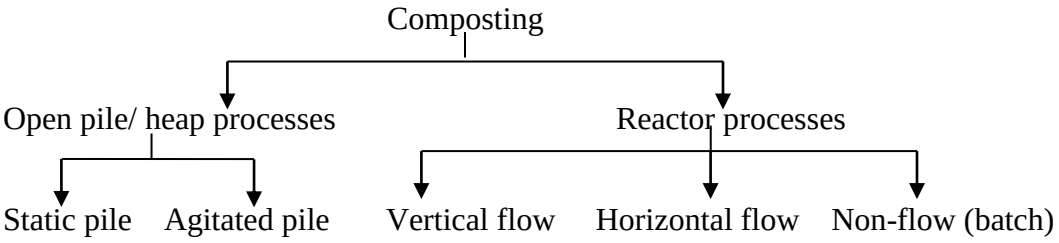
India is bestowed with wide range of agro-climatic conditions which are suitable for growing a wide range of horticultural crops such as flowers, fruits, nuts, spices and plantation crops. Due to this reason, the country has emerged as a major producer of fruits and vegetables in the world and contribution of the horticulture sector to Gross Domestic Product (GDP) from agriculture has been increasing over the years. It has been estimated that nearly 110-115 million tonnes of agro-waste in the form of crop residue and by products are generated per annum. This has the potential to be converted 0.7 Tg of good quality of compost every year. Such a massive quantum of compost is economically important in terms of crop nutrients besides innumerable benefits in terms of improvement of soil health and sustenance of crop productivity. But such a scenario remains distant dream due to the lack of efficient scientific strategies for bioconversion of these residues and lack of awareness amongst the farming community. Large scale burning of crop residues and improper disposal of byproducts have resulted in environmental pollution and ultimately led to the loss of precious plant nutrients. With a massive increase in horticultural crop production over the years, the quantities of residues and the byproducts have also seen a quantum jump leading to problems of efficient bioconversion and efficient disposal. The composting strategies that were developed earlier largely depended on the use of cattle manure as a source of compost starters besides serving as nutritional reservoirs for the microorganisms involved in the composting process. But with the rapid advent of urbanization the quantity of cattle manure available has drastically reduced in periurban areas, forcing us to seek alternative avenues. Therefore, the present study aims at identifying potential microbial agents that can be used for bioconversion of horticultural wastes in to nutrient rich composts.

Composting is the process of controlled microbial aerobic decomposition and stabilization of organic substrates to produce a stable end product. The term compost refers to the stable, humus-like product resulting from the biological decomposition of organic matter under controlled conditions. The main product of aerobic composting are carbon dioxide, water, mineral ions and stabilized organic matter (humus) (Gigliotti *et al*. 2005)

$$\text{Fresh organic waste} + O_2 \xrightarrow{\text{Microbial metabolism}} \text{Stabilized organic material} + CO_2 + H_2O + \text{heat}$$

Composting is accomplished through different phases *viz.*, the initial phase during which the readily degradable components are decomposed, the thermophilic phase during which cellulose and similar materials are degraded by a high bio-oxidative activity and the compost achieves a great degree of stability. The phases are conventionally defined in terms of kinds of bacterial and fungal populations that survive in different temperatures, *viz.*, psychrophilic, mesophilic and thermophilic (Dicson *et al*. 1991).

Composting systems can be categorized in to open to air systems and in-vessel systems. Open air systems are again categorized as static and agitated pile systems.



The static pile is the simplest and has the least operation and capital costs compared to all other methods of composting.

The composting process is influenced by both by biotic and abiotic factors. Abiotic factors include the nature of the substrate, Carbon/Nitrogen (C:N) ratio, moisture, oxygen, temperature, aeration, pH and Electrical Conductivity (EC). Biotic factors such as the population of bacteria, fungi and Actinobacteria play an important role in composting. Enzymes produced by these microbes play a key role in composting process. Traditionally cattle dung is used as a compost starter owing to its high microbial load and ability to sustain the population of the native microflora. But the rapid advent of urbanization has placed a constraint on the availability of cattle dung as a compost starter especially in peri-urban locations. Therefore this study was conducted with the following objectives.

1.2. Objectives

1. Isolation and selection of microbial bioconversion agents from horticultural ecosystem based on their bioconversion efficiency and amenability for consortia production
2. Characterization of elite microbial bioconversion agents and development of inoculants delivery systems
3. Evaluation of the formulated microbial conversion agents in association with different horticultural residues
4. Evaluation of the bio-enriched composts in tomato crop

CHAPTER-2

Review of Literature

REVIEW OF LITERATURE

Horticultural wastes are generally lignocellulosic in nature (Pandey *et al*. 2000). Lignocellulose is chemically composed of two linear polymers *viz*., cellulose and hemicellulose and a nonlinear polymer *viz*., lignin (Perez *et al.* 2002). Cellulose a linear polysaccharide is the most abundant component of plant biomass in nature (Lynd *et al*. 2002) and a major component of plant cell wall (Beguin and Aubert, 1994). The D-glucose subunits of cellulose are linked by β-1,4 bonds. Cellulases are enzymes which were involved in the biodegradation of cellulose. Three major components of cellulolytic enzymes include exoglucanases, endoglucanases and β-glucosidases. Bacteria, fungi and protozoa are the major cellulolytic enzyme producers. Alexander (1961) reported that aerobic and anaerobic mesophilic bacteria, filamentous fungi, thermophilic and alkalophilic bacteria, actinomycetes and protozoa are cellulose utilizers. Lynd *et al*. (2002) reported that the decomposition of organic matter especially cellulosic substances in particular was carried out by fungi. Cellulosic substances are broken down by cellulolytic bacteria and fungi (Fuller *et al*. 1943). Sharma *et al.* (2013) isolated *Bacillus subtilis*, *B.megaterium* and *B.licheniformis* which are potential cellulolytic and xylanolytic bacteria from composts. Uttam Kumar *et al*. (2014) reported that *Trichoderma viride* and *Aspergillus niger* isolated from forest wastes were potential cellulase producers. Cellulase producing bacteria such as *Bacillus cereus*, *B.tequilensis*, *B.safensis*, *B. altitudinis* and *Paenibacillus* were isolated and screened from oil palm meal on carboxy methyl cellulose agar (Khianngam *et al*. 2014). Imran *et al*. (2016) reported that *Trichoderma* sp., *Aspergillus* sp. and *Humicola* sp. were the important cellulase producers isolated from agricultural wastes. Cellulolytic activity has been reported in some bacterial genera such as *Cellovibrio*, *Pseudomonas* (Nakumura *et al*. 2006), *Cellulomonas*, *Bacillus* and *Micrococcus* (Immanuel *et al*. 2006). Kale *et al*. (2016) isolated and screened

cellulolytic fungi such as *Trichoderma reesei*, *Aspergillus flavus, Aspergillus fumigatus* from soil.

The second most abundant constituent of plant cell wall is hemicellulose which is composed of pentoses such as D-xylose and L-arabinose, hexoses such as D-mannose, D-glucose and D-galactose and also sugar acids (Sjostrom, 1993). Burlacu *et al*. (2016) reported that the second most abundant component of hemicellulose is xylan. Xylan has been categorized as arabinoxylan, glucurono xylan, galacto glucurono arabinoxylan and glucurono arabinoxylan. Xylan is a polymer of xylose (Polizeli *et al*. 2005). Xylanases are the enzymes that hydrolyze xylan. Various genera and species of bacteria, fungi and actinomycetes such as *Aspergillus* sp., *Penicillum* sp., *Trichoderma* sp., *Aureobasidium* sp., *Fusarium* sp. produce xylanases (Gupta *et al*. 2009) and xylanase production has been reported from *Rhizomucor* sp., *Humicola* sp., *Bacillus* sp., *Thermomonospora* sp., *Streptomyces* sp. (Sanghi *et al*. 2010; Annamalai *et al*. 2009; Kheng and Omar 2005). The hydrolytic enzymes secreted by fungi are extracellular with higher activities than yeast and bacteria (Adesina *et al*. 2016).Fungal xylanases have been reported by Mandal (2015), Haltrich *et al*. (1993), Haltrich *et al*. (1996) and Taneja *et al*. (2002) and Karunakaran *et al*. (2014) reported the production of xylanase from *Aspergillus niger* by submerged fermentation. Nair and Shashidhar (2008) reported that submerged fermentation and solid state fermentation process could be used for xylanase production. Sushir *et al*. (2016) isolated and screened the xylanolytic fungi, *Aspergillus heteromorphus*, from forest, farm soil and compost piles.

The most abundant and complex phenolic polymer of the plant cell wall is lignin which accounts for 15-36% of dry weight of wood. Lignin is a complex hetero-polymer that is usually complexed with cellulose. Lignin bio-degradation is mainly carried out by white rot and brown

rot fungi (Sanchez, 2009). These fungi produce extra cellular lignolytic enzymes like lignin peroxidases, manganese peroxidases and copper dependent laccases (Have *et al*. 2001). Several bacterial species *viz.*, *Rhodococcus erythropolis*, *Burkholderia* and *Citrobacter*, isolated from termite guts are also known to degrade lignin (Harazono *et al*. 2003). Ahmad *et al*. (2010) reported that the activity of lignin degrading bacteria was much less than those of white rot fungi.

Liers *et al*. (2006) reported that lignin and lignin model compounds were mineralized to some extent by wood-rotting Ascomycete species. Pointing *et al*. (2003) also reported that white rot-like decay and substantial lignin loss by *Xylaria s*pecies. Sasikumar *et al*. (2014) reported that lignin degrading microbes such as *Pseudomonas* sp. have been isolated from cow dung, soil compost and from paper pulp effluent and screened on Luria-Bertani (LB medium) containing methylene blue indicator dye. Laccases are multi-copper oxidoreductases that oxidize diphenol and other allied substances (Ducros *et al*. 1998). *Trichoderma atroviride*, *Trichoderma harzianum*, *Trichoderma longibrachiatum* are laccase producers isolated from farm soil, forest soil and bark scrapings (Desai *et al*. 2011). Christie *et al*. (2012) isolated laccase producing Ascomycetes species such as *Aspergillus niger*, *Penicillium marnefei*, *Alternaria arborescence* and *Fusarium oxysporium* on media containing ABTS and guaiacol. Kumar *et al*. (2011) reported that laccase producing fungal species such as *Agaricus bisporus*, *Fusarium solani*, *Pleurotus ostreatus*, *Pleurotus eous* and *Penicillium chrysogenum* could be used in decolourization of dyes.

Tannin is another important poly-phenolic compound found in abundance in vascular plants and is resistant to microbial attack (Bajpai *et al*. 1999). Tannins occur in hydrolysable form such as tannic acid, methyl gallate, ethyl gallate, isoamyl gallate. Tannase is the enzyme

which acts on tannin, is produced by micro-aerobic filamentous fungi such as *Trichoderma*, *Candida*, *Chaetomium*, *Fusarium*, *Rhizoctonia* and *Cylindrocarpon* (Saxena *et al*. 1995). Some fungi and a few bacterial species are able to break down tannins (Deschamp *et al*. 1983). Aoki *et al*. (1976), Bertolin *et al*. (2001) and Cavalitto *et al*. (1996) reported extracellular tannase production by bacteria, fungi and yeast. Tannase is produced by some bacterial species such as *Bacillus licheniformis* (Keshab *et al*. 2000). Deepanjali *et al*. (2012) reported the production of tannase from *Aspergillus niger* isolated from bark of *Acacia nilotica.*

The primary cell wall and middle lamellae of fruits and vegetables are made up of structural polysaccharides composed of pectin (Favela-Torres *et al*. 2012). Pectinases are enzymes that hydrolyze pectic substances (Gupta *et al*. 2008). Janani *et al*. (2011) isolated pectinase producing *Bacillus* sp. from an agricultural waste dump. Ramachandran *et al*. (2013) isolated *Penicillium citrinum* from fruit and vegetable wastes. Kaur *et al*. (2014) reported that pectinase produced by fruit spoilage fungi belongs to *Aspergillus* group. Banakar *et al*. (2012) isolated fungal species that produce pectinolytic enzymes from the forest soils of Western Ghats of Southern India. Varghese *et al*. (2013) isolated and screened pectinolytic microorganisms such as *Staphylococcus gelatini*, *Staphylococcus aureus*, *Streptococcus sp*., *Aspergillus sp*., *Rhizopus sp.* and *Mucor sp.* from fruit and vegetable dump soils of Raipur city.

Screening of microorganisms which are involved in biodegradation of crop wastes is carried out by culturing them on selective media. Cellulose degraders are usually screened on CMC agar medium (Bobbie and Leatherwood, 1976). Carboxy Methyl Cellulose (CMC) is used as sole source of carbon for the isolation of cellulolytic fungi such as *Aspergillus* sp. from castor bean waste (Polyanna *et al*., 2011). *Aspergillus fumigatus* a cellulase producing fungus was screened on Carboxy Methyl Cellulose agar plates by Lalithakumari *et al*. (2011). Mukhlis *et*

al.(2013) reported that the bioconversion of oil palm empty fruit bunches was carried out by lignocellulolytic *Trichoderma* sp. Rahna *et al*. (2012) reported the use of a selective media such as Carboxy Methyl Cellulose agar for the isolation of cellulolytic fungi from soil, compost and decayed lignocellulosic wastes. Girdhari and Peshwe (2015) isolated and screened tannin degrading fungi *viz.*, *Aspergillus fumigatus*, *Penicillium citrinum*, *Penicillium lividum* on tannic acid agar. Lignolytic fungi can be cultured on a defined liquid medium supplemented with 0-2 % lignosulfonic acid or tannic acid as sole carbon source, under micro-aerobic conditions (Silva *et al*. 2010). Screening of lignolytic microbes can also be carried out on Crawford's liquid medium containing alkali lignin (Crawford *et al*. 1982). Lignin degrading fungi can be screened by using synthetic phenolic reagents such as guaiacol and syringaldazine (Nishida *et al*. 1988) or polymeric dyes such as Remazol Brilliant Blue R (RBBR) and poly R-478 which are decolorized by lignin degrading fungi (Barbosa *et al*.1996; Gold *et al*. 1998). Singh and Abraham (2014) screened laccase producing fungi on PDA with an indicator compound. Guisado *et al*. (2012) isolated and screened the lignolytic fungi such as *Penicillium chrysogenum* and *Pseudallescheria angusta* from an compost environment by using polymeric dyes. Alfarra *et al*. (2013) reported that laccase producing fungi can be screened efficiently on media containing a chromogenic substance such as ABTS. Oyeleke *et al*. (2012) has reported that the pectinase producing fungi can be screened on Sabouraud's Dextrose Agar (SDA) containing pectin.

Compatibility between cultures plays an important role in development of a consortium. Sarkar *et al*. (2011) reported that bacterial cultures must be compatible with each other in order produce all the enzymes required for degradation of organic wastes. Rajasekhar *et al*. (2016) reported that a combination of microorganisms (mixed inoculants) interact synergistically and are found to be better than single inoculants.

Recent trends in arriving at the taxonomic position of bacteria have largely depended on sequence data of the 16S rRNA gene of bacteria (Carmen *et al*. 2006). But considering the limitations of this, polyphasic taxonomy that involves the biochemical, physiological and molecular data is preferred in order to arrive at a meaningful conclusion. For fungi, the nucleotide sequences of ribosomal RNA genes provide a wide range of taxonomic levels. Balajee *et al*. (2007), stated that the ITS primers have been designed to amplify the conserved regions of the 18S, 58S and 28S rRNA genes and the non-coding regions between them in order to arrive at a meaningful conclusion regarding the taxonomic position of fungi .

Composting of horticultural wastes is influenced by physical and chemical properties of the material. Composting is directly affected by the moisture content, aeration and temperature. Other factors like the particle size, moisture, nutrient content and quantity of organic residues have a pronounced effect on the composting process. Sundberg *et al.* (2013) reported that at low pH conditions, the rate of organic waste decomposition is slow. Liang *et al.* (2003) reported that the dominant factor in aerobic composting is the moisture content. Makan *et al.* (2012) reported that the moisture content maintains the temperature for longer time period and provides better degradation of organic matter. The temperature and microbial activity are inversely proportional to the moisture content of compost. Tiquia *et al.* (1996) reported that at the initial stage of composting process the moisture content is optimized and its value should not be beyond 50-60%. Moisture enhances the activity of microbes which further increases the rate of metabolism. At low moisture levels the activity of microbes is minimum. Composting of two or more materials together (co-composting) may accelerate the composting process, optimize C: N ratio, moisture content and particle size of the materials (Stratton and Rechcigl, 1997). Compost inoculants such as cellulolytic and lignolytic microorganisms like *Trichuris spiralis*,

Paeciliomyces, *Fusisporous*, *Trichoderma* and *Aspergillus* sp. are used for hastening of the composting process and improvement of final quality of end product (Gaur, 1982).

For successful application of compost its stability and maturity plays a vital role, especially for composts used in high value horticultural crops. Iannotti *et al*. (1993) reported that the lack of phytotoxicity indicates compost maturity whereas the microbial activity of the compost indicates its stability. Sa'nchez-Monedero *et al*. (1999) reported that the stability of compost is indicated by chemical parameters such as pH, electrical conductivity (EC), ratios of C to N and NH_4 to NO_3. Formation of humus like substances implies stabilization of compost. Stability of compost is determined by the humification index; however their absolute values vary greatly among composts for a long time the C/N ratio has been used as an index of compost maturity (Golueke, 1981). Other parameters have been proposed by different authors to monitor the composting process, among which the potential to degrade cellulose (Smith and Hughes, 2001), the Cation Exchange Capacity (Saharinen *et al*. 1996) or oxygen and CO_2 respirometry (Iannotti *et al*. 1994) can be mentioned.

Compost maturity generally refers to lack of toxins such as acetic acids, phenol and ammonia, stabilization of nutrients such as nitrate phosphorous, iron, the absence of detrimental bacteria, fungi, noxious odors and a noticeable reduction of heating upon rewetting (Stratton *et al*. 1995). According to Wang *et al*. (2004) understanding the changes that the material undergoes with the composting process is necessary to obtain high quality compost. The lack of a clear definition of maturity makes it difficult to evaluate when compost has reached this stability level. Wu *et al.* (2000) reported that the degree of decomposition of phototoxic organic substances produced during the active composting phase, absence of pathogens and viable weed seeds indicate compost maturity.

Many different parameters are involved in this process and some of them can be related to the changes that takes place in this phase. According to Zucconi and De Bertoldi (1987) a germination index higher than 60% compared to control with distilled water compost will be considered as mature.

CHAPTER-3

Materials and Methods

MATERIALS AND METHODS

3.1. General

3.1.1. Location

The laboratory and pot culture experiments were conducted in the Soil Microbiology Laboratory, Division of Soil Science and Agricultural Chemistry, ICAR- Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, which is located at an altitude of 890 meters above mean sea level, 13°N latitude and 77°E longitude. The pot culture studies were carried out during the period July 2014 -December 2014.

3.1.2. Agro-climatic conditions

The mean maximum and minimum temperatures that prevailed at Hesaraghatta, Bengaluru during the study period were 29.9°C and 21°C respectively. The mean relative humidity was 61.1 percent and the annual rainfall was 674.2 mm.

3.1.3. Glassware

All the glassware were soaked in chromic acid solution, washed thoroughly with tap water and finally rinsed with double distilled water prior to use.

3.1.4. Chemicals

The analytical reagent (AR) grade chemicals of BDH, Merck, Himedia, and Qualigens, were used for biochemical studies.

3.2. Survey for collection of samples and isolation of microbes

3.2.1. Sample Collection

Samples of well and partially decomposed organic residues were collected from peri-urban regions of Bengaluru, *viz.,* Rajanakunte (13,292˚N77.543˚E,), Nelamangala (135˚ N77.23˚E,), Shivkote (13.076˚N77.558˚E) and Kanakpura (12.25˚N77.417˚E) of Ramanagara district of Karnataka, India. The locations from where samples were collected included compost yards, Farm Yard Manure (FYM) pits and perishable commodities disposal sites (Plate 3.1). Compost and FYM samples were collected in polythene bags and stored at room temperature, while samples originating from vegetable and fruit disposal sites were collected in polythene bags and stored at 4°C until processing.

3.2.2. Isolation of microbes

Samples were serially diluted and spread plated on Nutrient agar (NA) and Potato Dextrose Agar (PDA) for bacterial and fungal isolation respectively. The fungal isolation plates were incubated at 27°C for 72 h and bacterial isolation plates at 30ºC for 24 h. Well differentiated bacterial and fungal colonies were purified, stored in slants under refrigerated conditions and used for further studies.

3.3. Qualitative screening of microbes for enzymatic activities

3.3.1. Qualitative screening of bacteria for cellulolytic activity

Individual exponentially grown bacterial cultures were spotted in the center of Carboxy Methyl Cellulose (CMC) agar plates, containing 0.1% CMC. Plates were incubated at 28°C for 48 hours and flooded with Gram's iodine (2.0g potassium iodide (KI) and 1.0g iodine in 300 ml of distilled water) for 3 to 5 minutes (Kasana *et al*. 2008).The appearance of distinct, clear and

(A)

(B)

Plate 3.1. Sample collection sites (A) Farm yard manure (B) Fruit based industry dumping site

prominent zones of clearance around the colonies were taken as an indication of their cellulolytic activity. The cellulolytic indices of the bacterial isolates were calculated by determining the ratio of clearance zone to the diameter of the colony.

3.3.2. Qualitative screening of fungi for cellulolytic activity

Individual fungal isolates were cultured on PDA and 8 mm discs of the well grown fungal colonies were placed in the centre of Mandel and Reese Medium containing 1% Carboxy Methyl Cellulose (CMC). The plates were incubated for 5-7 days at 27˚C and flooded with 0.25% Gram's iodine solution. Cellulose degradation around the colonies appeared as distinct, clear and prominent zones of clearance around the colonies. The cellulolytic indices of the fungal isolates were determined and expressed as the ratio between the diameter of the degradation halo to the diameter of the colony growth (Teather *et al*. 1982).

3.3.3. Qualitative screening of fungi for xylanolytic activity

A 8 mm fungal disc of individual fungal culture was placed in the centre of Xylan agar (Xylan Basal Medium (XBM) incorporated with 4% birchwood xylan and 1.6% w/v of agar) plates, which were subsequently incubated in the dark at 25ºC for 2-5 days. After incubation, the plates were flooded with 0.25% Gram's iodine and left undisturbed for 5 minutes. The excess stain was poured off and the agar surface was washed with distilled water. A yellow-opaque formation around the colonies indicated xylan degradation, while the undegraded xylan containing portions appeared as a blue or reddish purple color (Pointing, 1999). The xylanolytic indices were determined and expressed as the ratio between the diameter of the degradation halo and the diameter of the colony growth.

3.3.4. Qualitative screening of fungi for tannin degrading activity

Point inoculation of 8 mm hyphal discs of individual fungal cultures was carried out at the center of Tannic Acid Malt Extract Agar (TAMEA) containing 1% tannic acid and 3% agar. The plates were incubated at 37ºC for 7 days. After incubation, the plates were flooded with 1% ferric chloride solution. The clear zone formed around the fungal colonies was taken as an indication of tannin degrading activity (Bradoo *et al*. 1997) The tannase indices were obtained by calculating ratio between diameter of the degradation halo and the diameter of the colony growth.

3.3.5. Qualitative screening of fungi for pectinolytic activity

A 8 mm disc of individual fungal isolates was placed at the centre of Mineral Salt Agar (MSA) plates containing 1% pectin ($NaNO_3$-2.0g, KCl-.0.5g, $MgSO_4.7H_2O$-0.5 g, K_2HPO_4-1.0 g, $FeSO_4.7H_2O$-0.01 g, pectin-10g, Agar-20.0g, pH-6.8, distilled water- 1000 ml) (Reddy and Sreeramalu, 2012). The inoculated plates were incubated at 30°C for 5 days and flooded with Gram's iodine solution (2 g of potassium iodide and 1 g of iodine in 300 ml of distilled water).The development of a clear zone around the colonies was taken as an index of pectin degradation (Soares *et al*. 1999). The pectinolytic indices were obtained by calculating the ratio between diameter of the degradation halo and the diameter of the colony growth.

3.3.6. Qualitative screening of fungi for lignolytic activity

3.3.6.1. Bavendamm test for overall polyphenol oxidase activity

The fungal cultures were subjected to the qualitative determination of the overall polyphenol oxidase activity which is not specific to any Lignin Modifying Enzyme (LME).

Lignin Basal Media (LBM) supplemented with 1% tannic acid was prepared and inoculated with a 8 mm disc of the test fungus. Plates were incubated at 25ºC in darkness for 10 days, and examined daily for the appearance of a brown oxidation zone around colonies which is indicative of total polyphenol activity of the test fungus (Pointing, 1999).

3.3.6.2. Qualitative determination of lignin peroxidase and manganese peroxidase activities

Lignin Basal Medium (LBM) supplemented with 0.01% (w/v) Azure B and 1.6% agar was prepared separately to which 1 ml of a sterile 20% (w/v) of aqueous glucose solution was added. This medium was inoculated with a 8 mm disc of test fungus and incubated in the dark at 25ºC for 10 days. The clearing zone formed around the colonies in the blue coloured medium was indicative of lignin peroxidase and manganese peroxidase activities (Pointing, 1999).

3.3.6.3. Qualitative determination of laccase activity

Lignin Basal Medium (LBM) supplemented with 0.1% (w/v) ABTS (2, 2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) and 1.6% (w/v) agar was prepared separately to which1 ml of sterile 20% (w/v) of aqueous glucose solution was added. This medium was inoculated with 8 mm disc of the test fungus and incubated in the dark at 25ºC in for 10 days. Qualitative determination of the laccase activity was recorded by visually recording the change in the colour of the medium from colorless to green due to the oxidation of ABTS to ABTS-azine in the presence of laccase (Pointing, 1999).

3.4. Quantitative enzyme assays

3.4.1. Assay for cellulase activity

Growth and culture conditions of bacterial and fungal isolates for cellulase enzyme assay

One ml of the exponentially grown cultures of individual elite cellulolytic bacterial isolates *viz.*, CB-7, CB-9, CB-16, CB-17, FLCB-5 and FLCB-11 were inoculated in CMC broth containing 0.1% CMC (Plate 3.2) and incubated at 30ºC for 48 h. At the end of incubation period the cell biomass was harvested by centrifugation at 8,000 rpm for 30 minutes, and the cell free extracts were used as crude enzymes for estimation of the cellulolytic activity of bacteria by the filter paper assay. For fungi a 8 mm disc of elite fungal isolates *viz*., CF-20, TMLF-1, PMLF-1 and FCLF-1 selected from the earlier qualitative screening process, was inoculated in Mandel and Reese broth containing 1% CMC (pH 5.6) (Plate 3.2) and incubated at 30ºC for 7 days. After incubation, the contents of the flasks were passed through a Whatman filter paper No.1 to separate the mycelia mat from culture filtrate. The filtrate thus obtained was used for the estimation of cellulase activity by the filter paper assay.

Filter Paper Assay (FPA)

The filter paper assay as described by Mandels *et al*., (1976) was performed. An aliquot of 0.5 ml of cell free culture supernatant was transferred to a clean test tube and 1ml of 0.05M sodium citrate buffer (pH 4.8) was added. A Whatman No.1filter paper strip (6 cm x 1 cm) was added to each tube. The tubes were vortexed to coil the filter paper in the bottom of tube. Tubes were incubated in a water bath at 50˚C for 1hour for the liberation of reducing sugars, followed by an addition of 3,5-dinitrosalicylicacid (DNS) reagent (3ml). Tubes were then placed in a

boiling water bath for 5 min and cooled in an ice bath, followed by the addition of 15 ml of distilled water to each tube. The contents of the tubes were mixed and absorbance was noted at 550 nm. The celluase activity was expressed in term of filter paper unit (FPU) per ml of undiluted culture filtrate. A filter paper unit (FPU) is defined as mg of reducing sugar liberated in one hour under standard assay conditions. The reducing sugar liberated was quantified using glucose as a standard (Ghose, 1987)

3.4.2. Assay for xylanase activity

The elite fungal isolates that showed significant xylanase activity *viz*., CF-11, VCLF-1, CP-2 and CF-6 were further subjected for quantitative estimation of their xylanolytic activity. Xylan broth (100ml) amended with 1% birchwood xylan, was prepared in 250 ml Erlenmeyer flasks. The pH of the medium was adjusted to 5. Flasks were incubated at 32˚C for 7 days. After incubation, the contents of the flasks were passed through Whatman filter paper No.1 to separate mycelia mat from culture filtrate. The filtrate thus obtained was used for the estimation of xylanase activity according to method described by Bailey *et al*. (1992). A reaction mixture consisting of 1.0 ml of crude enzyme sample, 1 ml of birchwood xylan (prepared in 0.05M Na-citrate buffer, pH 5.3) and 1 ml of 0.05M citrate buffer were incubated at 55°C for 10 min.The reaction was stopped by addition of 3.0 ml of 3, 5-dinitro salicylic acid (DNS) and the contents were boiled for 15 min. After cooling to room temperature the intensity of the colour developed was read at 540 nm. The reducing sugar liberated was quantified using xylose as standard. One unit of xylanase activity was defined as the amount of enzyme that liberates 1 µmol xylose equivalent per minute under standard assay conditions.

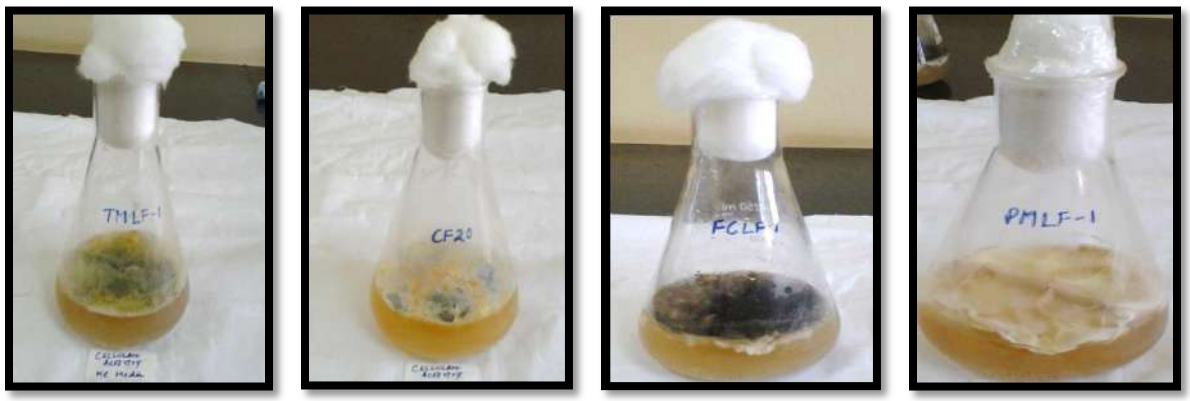


Plate 3.2. Fungal cultures inoculated in MR broth for determination of cellulase activity

Plate 3.3. Fungal cultures inoculated in Tannic Acid Malt Extract Broth for determination of tannase activity

3.4.3. Assay for tannase activity

The elite isolates that showed significant tannase activity *viz*., CF-11, VCLF-1, CP-2 and CF-6 were further subjected for quantitative estimation of their tannase activity. Tannase assay was carried out by estimation of gallic acid by HPLC. Malt extract broth (250 ml) amended with 1% tannic acid was prepared in 250ml Erlenmeyer flasks (Plate 3.3). Flasks were incubated at 32°C for 3-5days. After incubation, the fungal mat was broken with a magnetic stirrer and the fungal culture was filtered through a Whatman No.1 filter paper under ice cold conditions. This filtrate was used for enzyme analysis. One ml of enzyme filtrate was made up to 25 ml with distilled water. Twenty µl of this filtrate was injected in to a HPLC. Gallic acid was used as standard. The peak and retention times obtained were compared with a gallic acid standard as descried by (Paranthaman *et al*. 2009)

3.4.4. Assay for pectinase activity

Eight mm discs of elite fungal isolates *viz*., VCLF-1, FLF-11 and LG-1 selected from the earlier qualitative screening process were inoculated in a Mineral Salt Broth containing 1% pectin and incubated at 30°C for 7 days. At the end of the incubation period, the cell biomass was separated by filtration by using a Whatman No. 1 filter paper under ice cold conditions. This filtrate was used as crude enzyme for estimation of the pectinase activity. Quantitative estimation of pectinase activity was performed by the DNS method (Miller, 1959), with pectin as substrate. A cell free suspension of 0.5 ml was inoculated with 0.5 ml of pectin in 0.1M acetate buffer at a pH 6.0 and the reaction mixture was incubated at 40°C for 10 minutes under static conditions. After adding 1ml of DNS reagent, the mixture was boiled for 5 min at 90˚C. The reaction was stopped by adding 1ml of Rochelle's salt. To this 2 ml of de-ionized water was added to dilute

the mixture and the absorbance was read at 540 nm. One unit of pectinase activity was defined as the amount of enzyme which liberated 1µm glucose per min (Janani *et al*. 2011). A glucose standard curve was used to quantify the glucose liberated in the reaction mixture.

3.5. Molecular identification of elite isolates

The selected bacterial and fungal isolates were purified on selective media and inoculated in broth cultures for genomic DNA isolation. Bacterial and fungal cells were harvested either by centrifugation or filtration. The genomic DNA of both bacteria and fungi was isolated by using the respective DNA isolation kit (Zymogen, India). The identity of the organisms were arrived by the sequencing of the amplified 16S rRNA region of bacteria and the internally transcribed spacer (ITS) region for fungi as described by White *et al*. (1990). The universal primers 27F and 1525R were used to amplify the 16S rRNA region of bacteria and the universal primers ITS1 and ITS4 were used to amplify the ITS region of fungi. The sequence reads subjected to a BLASTn analysis in the NCBI database and the highest percent identity was used to arrive at a conclusion on the identity of the individual organisms.

3.6. *In vitro* screening studies for evaluating the humification potential of the elite cultures using chilli stalks as a substrate

Fifty gm of finely chopped dried chilli stalks were taken in a 50 ml of plastic beaker and moistened with 60 ml of sterile distilled water. To this moistened sample, 1 ml of 24 h old culture of bacterial cultures and 72 h old fungal spore suspensions of individual cultures were inoculated and incubated for 49 days at room temperature with periodic moistening in order to maintain the moisture content of the substrate at 50% (w/w). At the end of the incubation period,

the Humification Index (HI), of the partially decomposed chilli stalk substrates were analyzed as detailed below.

3.6.1. Determination of the Humification Index (HI)

One gm of partially decomposed chilli stalks samples were taken in 100 ml plastic vials, to this 50 ml of 0.5M NaoH was added and placed on a rotary shaker for 2 h and incubated overnight at room temperature. Subsequently the contents were filtered through a Whatman No.1 filter paper and the absorbance of the filtrate was measured at 472 nm and 664 nm (Sapek and Sapek, 1999). The absorbance ratio of $Q_{4/6}$(472nm/664nm) indicates the degree of humification of the substrate.

3.7. Evaluation of compatibility among different isolates

Compatibility among selected isolates is an important criterion in the development of a consortium. The compatibility between selected isolates was determined by dual culture method technique (Dennis and Webster, 1971) and cross streak method. Compatibility between bacterial/fungal isolates and fungal/fungal isolates was determined on Potato Dextrose Agar (PDA). The compatibility between fungal cultures was checked by placing 8 mm fungal discs of the test fungi on either sides of a Petri plate (1 cm from edge of the plate) and incubating at 27°C. The compatibility between the bacterial and fungal cultures was determined by streaking the test bacterial strain on one side (1 cm from edge of the plate) and placing the test fungi (8 mm disc) was on opposite side of the same plate and incubating at 27°C. The absence of a zone of separation between the test isolates was taken as a measure of the compatibility amongst them.

3.8. Formulation of lignite based compost starters

For formulation of lignite based compost starters, individual bacterial and fungal cultures were cultured on nutrient broth and potato dextrose broth separately and mixed with carrier material *viz.*, lignite 30% (v/w) under sterile conditions. The lignite based formulations were cured for twenty four hours and stored in LDPE bags for further use.

3.9. Development of substrate specific compost starters

Based on the compatibility among elite isolates and their functional properties three consortia comprising equal proportions of lignite based compost starters of individual isolates with different functional traits were formulated (Table 3.1).

Table 3.1. Compost starter consortia used for studies on bioconversion of horticultural crop residues

Consortium -1	Consortium-2	Consortium-3
Aspergillus sp. TMLF-1 (Cellulolytic)	*Bacillus endophyticus* FLCB-11 (Cellulolytic)	*Penicillium citrinum* CF-20 (Cellulolytic)
Aspergillus oryzae CP-2 (Xylanolytic)	*Aspergillus* sp. CF-11 (Xylanolytic)	*Alternaria tenuissima* LG-1 (Xylanolytic)
Trichoderma hamatum FLF-13 (Lignolytic)	*Xylaria* sp. TF-4 (Lignolytic)	*Aspergillus affustus* FLF-11 (Lignolytic)
Aspergillus sp.TL-8 (Tannin Degrading)	*Penicillium chrysogenum*VCLF-1 (Tannin Degrading)	*Aspergillus* sp. TL-8 (Tannin Degrading)

3.10. Compost production studies using the compost starter consortia in conjuction with different horticultural residues

The bioconversion efficacy of the three consortia was determined in conjuction with five different horticultural residues *viz.*, brinjal stalks (semi-succulent), chilli stalks (woody), *Dolichos* stover (succulent), grape pruning's (woody) and mango leaf litter (litter) in pots under glass house conditions. Pots of dimension 30cm x 26cm x 22 cm length were filled with 500 g of respective substrates and the lignite based compost starters (5% w/w of substrate). Nitrogen was supplied to the pots as urea (1% w/w of substrate) and the moisture content of composting mass was maintained at 50-60% (w/w). The pots were covered with black plastic sheets to avoid heat and water loss. The material in the pots were turned at regular intervals to provide aeration. The moisture content was maintained during each turning by addition of water. The experiment was carried out in the completely randomized design with four treatments and four replications for a period of 90-150 days depending on the nature of the substrate used. Periodical observations on compost stability and maturity parameters were undertaken during this period.

3.11. Evaluation of prepared composts for their stability and maturity

At the end of the study period the prepared composts were evaluated for their stability and maturity using different parameters *viz.*, pH, Electrical Conductivity (EC), moisture content, Humification Index (HI), carbon to nitrogen (C:N) ratio, contents of Total Organic Matter (TOM), Total Organic Carbon (TOC), Water Soluble Carbon (WSC),Water Soluble Nitrogen (WSN), Humic like Carbon (HC), Fulvic like Carbon (FC), Alkali Extractable Carbon (C_{ext}) as per standard methods (Paradelo *et al*. 2013). The phytotoxic potential of the composts were determined as suggested by Zucconi *et al*. (1985) using radish seeds.

3.11.1. pH and Electrical Conductivity (EC)

The pH and Electrical conductivity (EC) of the prepared composts were analyzed in the water extracts (1:10 w/v) with an electronic pH/EC meter (PCS Testr 35 S/N: 1430653).

3.11.2. Moisture content

The moisture content of compost samples was determined by drying in a moisture box at 105°C in an oven for 24 h (Bazrafshan *et al*. 2016). The empty weight of moisture box with lid was recorded as W1, the weight of moisture box with sample before drying was recorded as W2 and the weight of the moisture box with sample after drying was recorded as W3.

$$\text{Moisture Content (\%)} = \frac{W_2 - W_3}{W2 - W1} \times 100$$

3.11.3. Carbon: Nitrogen (C: N) ratio

The carbon to nitrogen ratio of the composts sample was determined in a CHNS analyzer by following the principle of complete combustion of the substrate and detecting the oxides of the individual elements using a thermal conductivity detector. Individual compost samples (10-20 mg) were weighed accurately in tin boats and packed carefully. The boats were fed into the auto analyzer (CHNS Elementar Vario Microcube), and the total carbon and nitrogen contents were determined by direct combustion of the accurately weighed samples at 1150°C.

3.11.4. Humification Index (HI)

One gm of each of the compost sample were taken in 100 ml plastic vials, to this 50 ml of 0.5M NaoH was added and placed on a rotary shaker for 2 h and incubated overnight at room

temperature. Subsequently the contents of the vials were filtered through a Whatman No.1 filter paper and the absorbance of the filtrate was measured at 472 nm and at 664 nm (Sapek and Sapek, 1999). The absorbance ratio of $Q_{4/6}$ (472nm/664nm) indicates the degree of humification.

3.11.5. Total Organic Matter (TOM) and Total Organic Carbon (TOC)

Total Organic Matter (TOM) of composts was determined by estimating the weight loss on ignition of one gm of dried and ground samples at 550°C for 5 h (Nelson and Sommers, 1996) in a muffle furnace. The TOM concentration of compost was calculated from the ash content:

$$\text{Ash content (\%)} = \frac{\text{Ash weight of compost (g)}}{\text{Dry weight of compost (g)}}$$

TOM content (g/kg) = 1000- Ash content of compost (%)

The total organic carbon was calculated by multiplying the organic matter concentration by a factor 0.58.

3.11.6. Water Soluble Carbon (WSC) and Water Soluble Nitrogen (WSN)

Preparation of compost samples

Aqueous extracts of the composts were prepared by the shaking of oven dried composts with distilled water in the ratio 1:10 (w/v) for 2 h, in a rotary shaker. The suspension was then centrifuged at 10,000 rpm for 30 min followed by filtration through a Whatman No. 42 filter paper (Hue and Liu, 1995). This aqueous extract was used for estimation of WSC and WSN of the compost samples.

Water Soluble Carbon (WSC)

Water Soluble Carbon content in the compost samples was determined by wet dichromate oxidation method as described by Walkley and Black (1934). Ten ml of 1N potassium dichromate and twenty ml of concentrated sulphuric acid were added to 0.1 ml of compost extracts and incubated for 30 min at room temperature. After incubation, 30 ml of distilled water was added and titrated against 0.5 N Ferrous Ammonium Sulphate (FAS) until the colour changed to green. Ferroin was used as an indicator.

$$\text{Water Soluble Carbon (\%)} = \frac{B-T\times 0.5\times 0.003\times 1000}{\text{Amount of sample taken}}$$

Where,

B – The titre value of blank

T – The titre value of test sample

0.5 – Normality of FAS

0.003– Black's constant

Water Soluble Nitrogen (WSN)

The water soluble nitrogen content of the compost extracts was determined by the Kjeldhal method (Bremmer, 1996). One g of compost sample was mixed with 20 ml of distilled water in a centrifuge tube and centrifuged at 10,000 rpm for 15 min and the resulting supernatant was used for the water soluble nitrogen estimation. Five ml of the supernatant was taken in a

distillation flask and 10 ml of 4% boric acid was taken in a receiving flask. Eight ml of 40% NaOH was added to the distillation flask and the contents were distilled. After complete distillation, the receiving flask was disconnected and contents of the receiving flasks were titrated against 0.05 N H_2SO_4 till the development of pink colour. From the burette reading the water soluble nitrogen content of the prepared compost extracts were calculated, using the formula

$$\text{Water soluble nitrogen (\%)} = \frac{\text{TV} \times \text{N of acid X } 0.014 \times \text{Vol. of digested sample}}{\text{Weight of sample} \times \text{Aliquot taken}} \text{ X } 100$$

Where,

TV- Titre value**;** N- Normality of sulphuric acid

3.11.7. Alkali Extractable Carbon (C_{ext})

The alkali extractable carbon was estimated from the compost extracts by mixing 1 gm of finely ground compost samples with 20 ml of 0.1 M NaOH and centrifuging at 10,000 rpm for 15 min. The clear supernatant so obtained contains the alkali extractable carbon. The alkali extractable carbon was estimated by wet dichromate oxidation procedure as detailed by Walkey and Black (1934) and described in greater detail in section 3.11.6.

3.11.8. Humic like Carbon (HC) and Fulvic like Carbon (FC)

The humic like carbon and fulvic like carbon fractions were extracted from alkali extractable carbon extract. The fulvic like carbon was separated by acidification with concentrated sulphuric acid till the pH dropped to 1.The extract was centrifuged at 10,000 rpm for 15 min and the supernatant so obtained was used for the fulvic like carbon estimation. The

precipitate obtained after centrifugation was dissolved in 2 ml of 0.5 M NaOH and adjusted to 50 mL with distilled water, and used for the estimation of humic like carbon (Sanchez-Monedero *et al*. 1996). The wet dichromate oxidation procedure of Walkey and Black (1934) was used for the estimation of the oxidizable carbon in both the fractions as described earlier in section 3.11.6.

3.11.9. Compost Maturity Parameters

3.11.9.1. Percent germination of radish seeds for determination of phytotoxicity

The maturity of the prepared composts was determined by the phytotoxicity test. An aqueous extract of the individual composts was obtained by shaking a compost-distilled water suspension (1:10 w/v) for 1 h and filtering the resultant extract. A filter paper was placed in a Petriplate and ten ml of the individual compost extracts were applied to the filter paper, ten seeds of radish (*Raphanus sativus)* were then placed on the filter paper. The experiment was carried out in triplicate (Plate 3.4). A control treatment with distilled water was also maintained and all the plates were incubated in the dark for 72 h at room temperature. The seed germination percentage was calculated using the formula given below.

$$\text{Percent germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \text{X100}$$

3.11.9.2. Direct growth test

A direct growth test using radish seeds was carried out to further evaluate the composts for the presence of phytotoxic components. Individual plastic pots were filled with a soil-test compost mixture in the ratio 1:1 (w/w) and four radish seeds were sown in each pot, watered and allowed to germinate in the dark at 30°C.

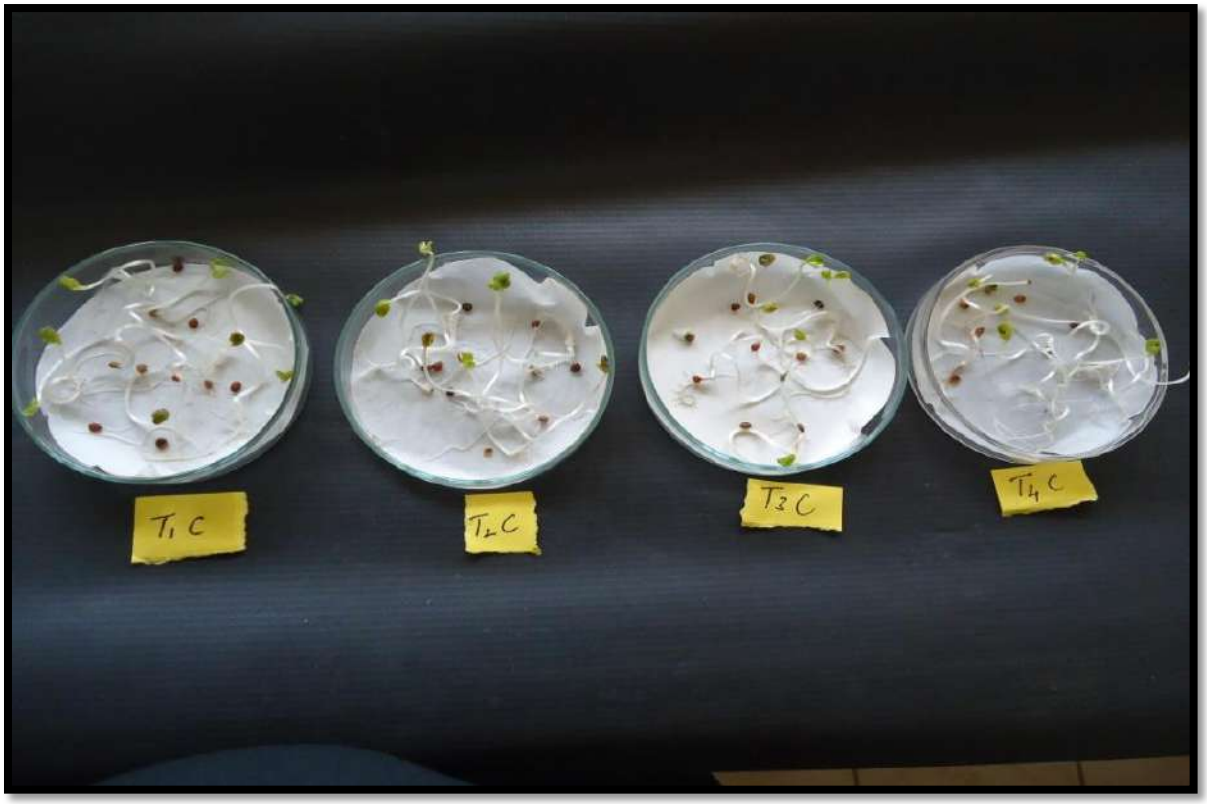


Plate 3.4. Seed germination assay with aqueous extracts of the prepared composts for determination of the phytotoxic potential of the composts

A control containing only soil was also used. The number of sprouts visible in each cup was counted daily for seven days after sowing. The percent germination of the was calculated using the number of seeds germinated and the number of seeds sown. The germination index (GI) was calculated as described by Zucconi *et al*.(1985).Where G and L are the germination and radical growth of the samples, respectively, and Gc and Lc are the germination and radical growth of the control (sterile distilled water), respectively.

$$GI = \frac{G}{G_C} \cdot \frac{L}{L_C} X100.$$

3.12. Evaluation of composts on tomato under polyhouse condition

The evaluation of the prepared composts on tomato hybrid UNIK-38 was carried out under polyhouse condition in plastic pots (14 inch dia), in a Completely Randomized Design. Individual pots were filled with 12 kg of soil and transplanted with twenty one day tomato seedlings. Individual oven dried compost samples (5.3 g), were diluted in water and applied in the root zone of the tomato seedlings. The seedlings were watered regularly and necessary plant protection measures were undertaken periodically. The vegetative parameters such as number of flowers and harvest parameters such as number of fruits and weight of fruits were recorded. The vegetative parameters such as number of flowers were recorded on 45^{th}, $60^{th,}$ 75^{th} and 90^{th} days of transplantation. The harvest parameters such as number of fruits and weight of fruits were recorded on the 75^{th} and 90^{th} day of transplantation.

CHAPTER-4

Results

RESULTS

4.1. Isolation of microbes

A total of twenty five bacterial and seventy fungal cultures were isolated from the samples originating from compost yards, Farm Yard Manure (FYM) pits and related environments on potato dextrose agar and on nutrient agar respectively (Plate 4.1).

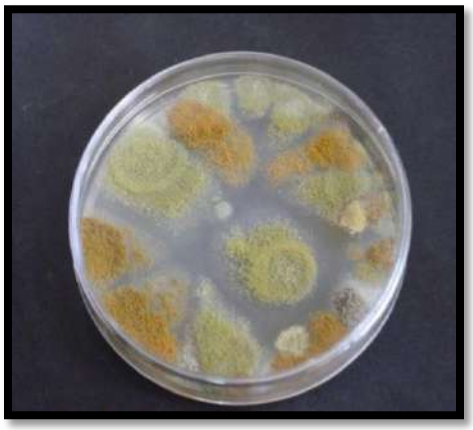
4.2. Screening of different isolates for enzymatic activities

4.2.1. Qualitative screening of bacteria for cellulolytic activity

Out of twenty five bacterial isolates, six bacterial isolates *viz*., *Bacillus cereus* CB-7, *Bacillus* sp. CB-9, *Bacillus invictae* CB-16, *Bacillus tequilensis* CB-17, *Bacillus simplex* FLCB-5 and *Bacillus endophyticus* FLCB-11 showed appreciable cellulolytic activities, with cellulolytic indices ranging from 6-8 on CMC agar (Figure 4.1 and Table 4.1). The isolate *Bacillus endophyticus* FLCB-11 showed the highest cellulolytic index of 8 on Carboxy Methyl Cellulose (CMC) agar followed by *Bacillus tequilensis* CB-17 and *Bacillus cereus* CB-7 (Plate 4.2).

4.2.2. Qualitative screening of fungi for cellulolytic activity

Out of seventy fungal isolates screened four fungal isolates *viz., Penicillium citrinum* CF-20, *Aspergillus sp.*TMLF-1, *Aspergillus sp*. PMLF-1, *Aspergillus sp*. FCLF-1 (Figure 4.2), showed significant cellulolytic activity on Mandel and Reese media with cellulolytic indices ranging from 1.2-1.6. *Penicillum citrinum* CF-20 (Plate 4.3) showed highest cellulolytic index of 1.6 on Mandel and Reese agar (Table 4.2).



(A) **(B)**

(C)

Plate 4.1. (A) Isolation of fungi on Potato Dextrose Agar **(B)** Isolation of fungi on Czapek Dox agar **(C)** Isolation of bacteria on nutrient agar

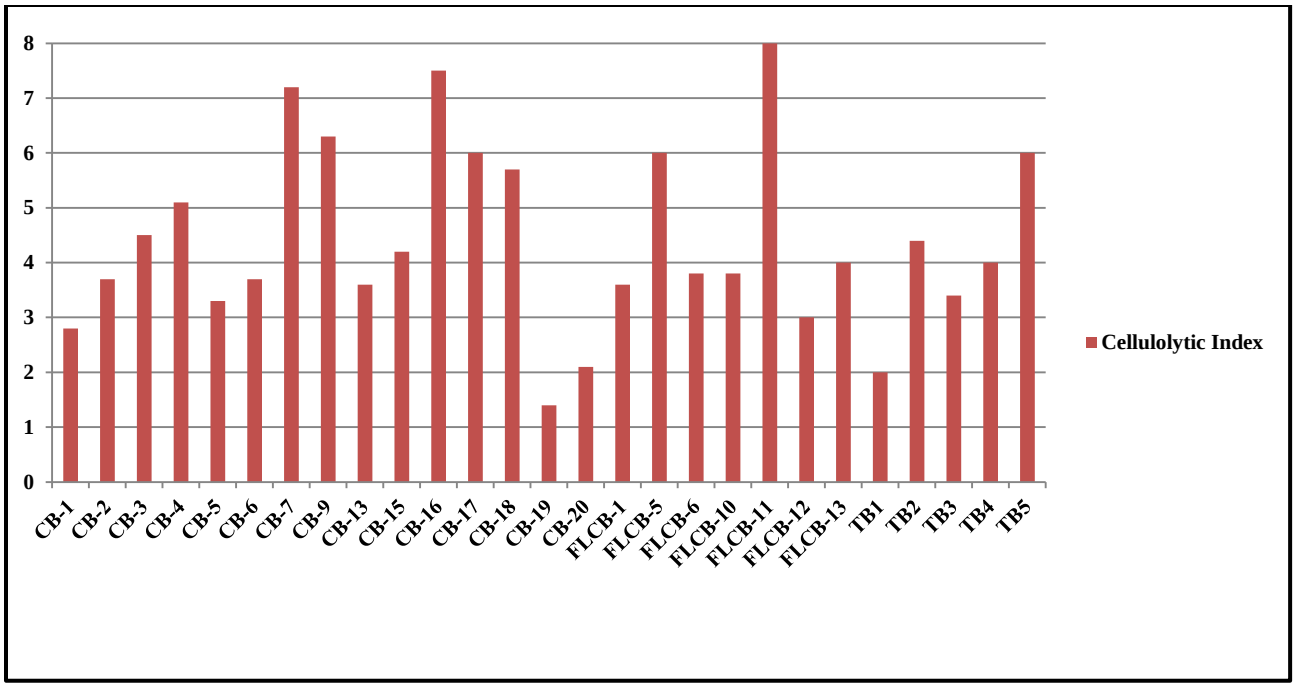


Figure 4.1. Cellulolytic indices of bacterial isolates

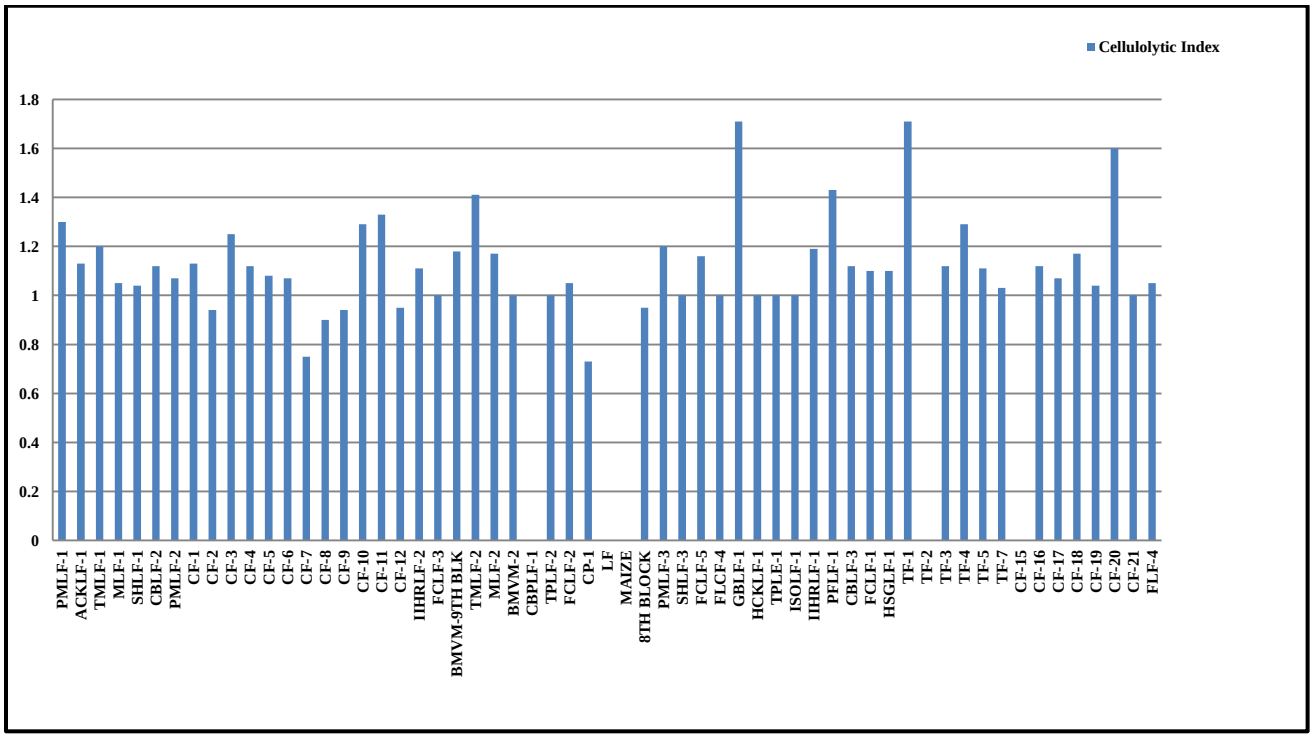
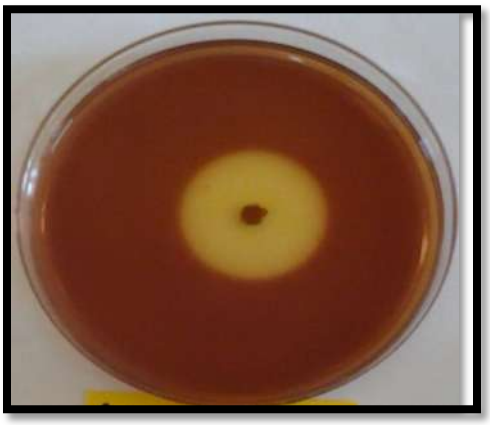


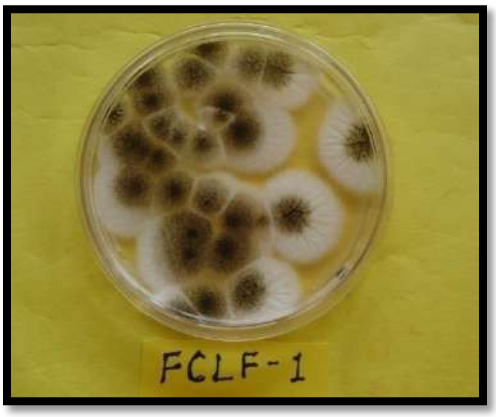
Figure 4.2. Cellulolytic indices of fungal isolates



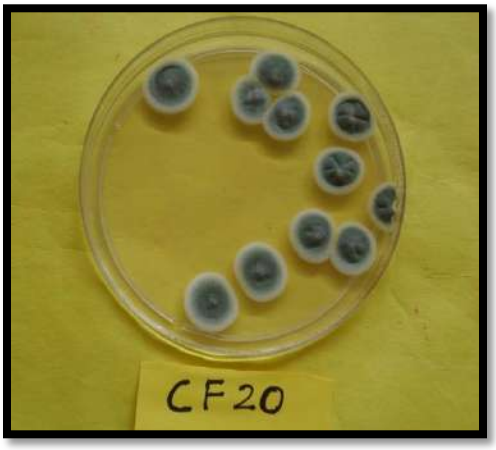
(A)

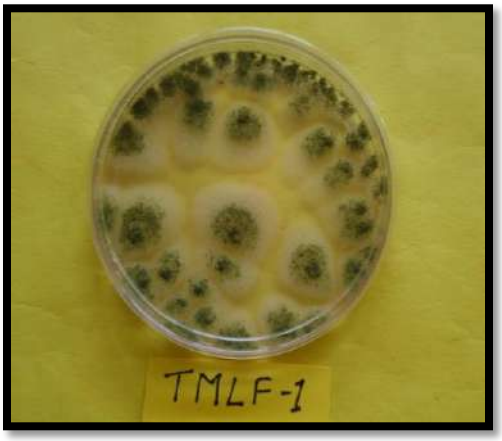
(B) **(C)**

Plate 4.2. (A) Cellulolytic activity of bacteria on CMC agar (**B)** Elite cellulolytic bacterial isolates *Bacillus cereus* CB-7 **(C)** *Bacillus endophyticus* FLCB-11



(A)





(B) **(C)**

Plate 4.3. Elite cellulolytic fungal isolates **(A)** *Aspergillus* sp. FCLF-1 **(B)** *Penicillium citrinum* CF-20 **(C)** *Aspergillus sp.* TMLF-1

Table 4.1. Cellulolytic indices of elite bacterial isolates

Name of organism	Cellulolytic index
Bacillus cereus CB-7	7.2
Bacillus sp. CB- 9	6.3
Bacillus invictae CB-16	7.5
Bacillus tequilensis CB-17	6.0
Bacillus simplex FLCB- 5	6.0
Bacillus endophyticus FLCB -11	8.0

Table 4.2. Cellulolytic indices of elite fungal isolates

Name of organism	Cellulolytic index
Penicillium citrinum CF-20	1.6
Aspergillus sp. TMLF-1	1.3
Aspergillus niger PMLF-1	1.2
Aspergillus sp. FCLF-1	1.1

4.2.3. Qualitative screening of fungi for xylanolytic activity

Out of seventy fungal isolates screened four fungal isolates *viz*., *Aspergillus sp.*CF-11, *Penicillium chrysogenum* VCLF-1, *Aspergillus oryzae* CP-2 and *Aspergillus sp.* CF-6 showed significant xylanolytic activity on xylan agar (Figure 4.3) with xylanolytic indices ranging from 1.7-1.3 (Figure 4.8). *Aspergillus sp*. CF-11 showed highest xylanolytic index of 3 on xylan agar plates (Table 4.3 and Plate 4.4).

4.2.4. Qualitative screening of fungi for tannin degrading activity

Out of seventy fungal isolates three fungal cultures *viz*., *Aspergillus fumigatus* CF-17, *Penicillium chrysogenum* VCLF-1 and *Aspergillus* sp. TL-8 showed tannase indices ranging from 2-3.5 on Tannic Acid Malt Extract Agar (TAMEA) (Figure 4.5 and Plate 4.6). The isolate *Aspergillus* sp. TL-8, showed highest tannase index of 3.5, followed by *Penicillium chrysogenum* VCLF-1 (Table 4.4).

4.2.5. Qualitative screening of fungi for pectinolytic activity

Out of seventy fungal isolates screened three fungal isolates *viz*., *Penicillium chrysogenum* VCLF-1, *Aspergillus affustus* CF-11 and *Alternaria tenuissima* LG-1 (Figure 4.4) possessed a pectinolytic indices ranging from 1.7-2 on MSA plates (Plate 4.5 and Table 4.5).

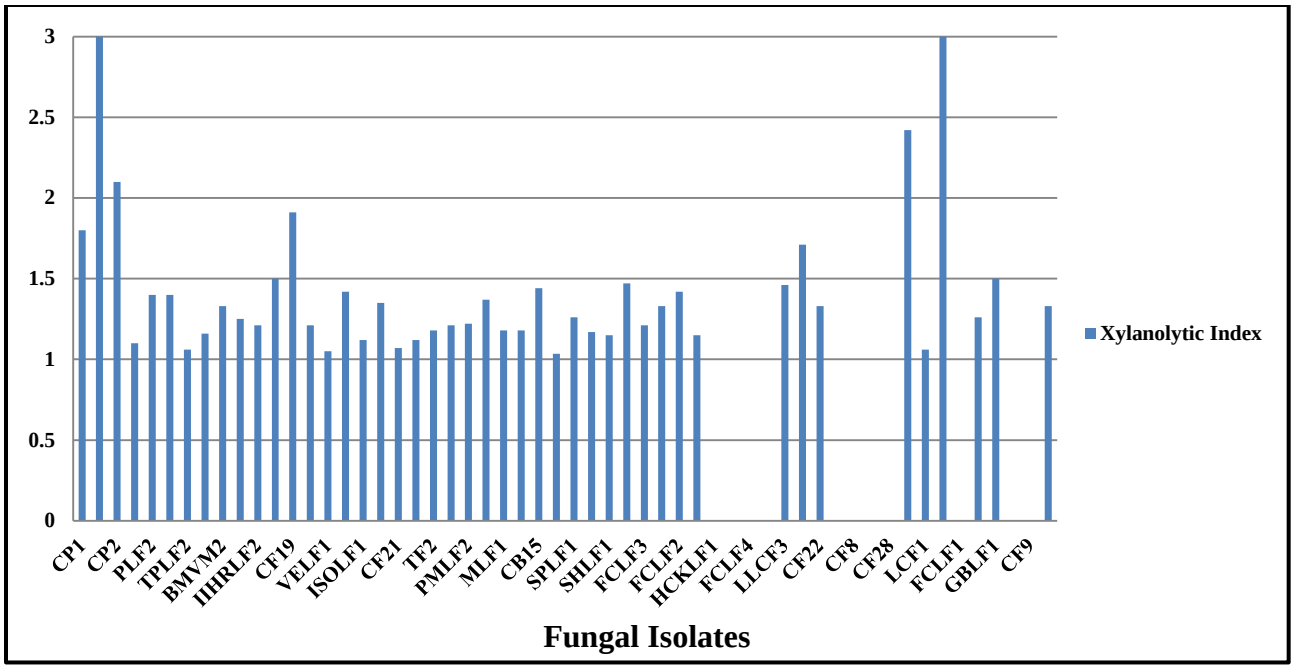


Figure 4.3. Xylanolytic indices of fungal isolates

Table 4.3. Xylanolytic indices of elite fungal isolates

Name of organism	Xylanolytic index
Aspergillus sp. CF-11	3.0
Penicillium chrysogenum VCLF-1	1.7
Aspergillus oryzae CP-2	2.1
Aspergillus sp. CF-6.	2.4

(A)

(B)

Plate 4.4. (A) Xylanase activity of elite fungal isolates on Xylan agar (**B)** Elite xylanolytic fungal isolate *Aspergillus sp.* CF-11

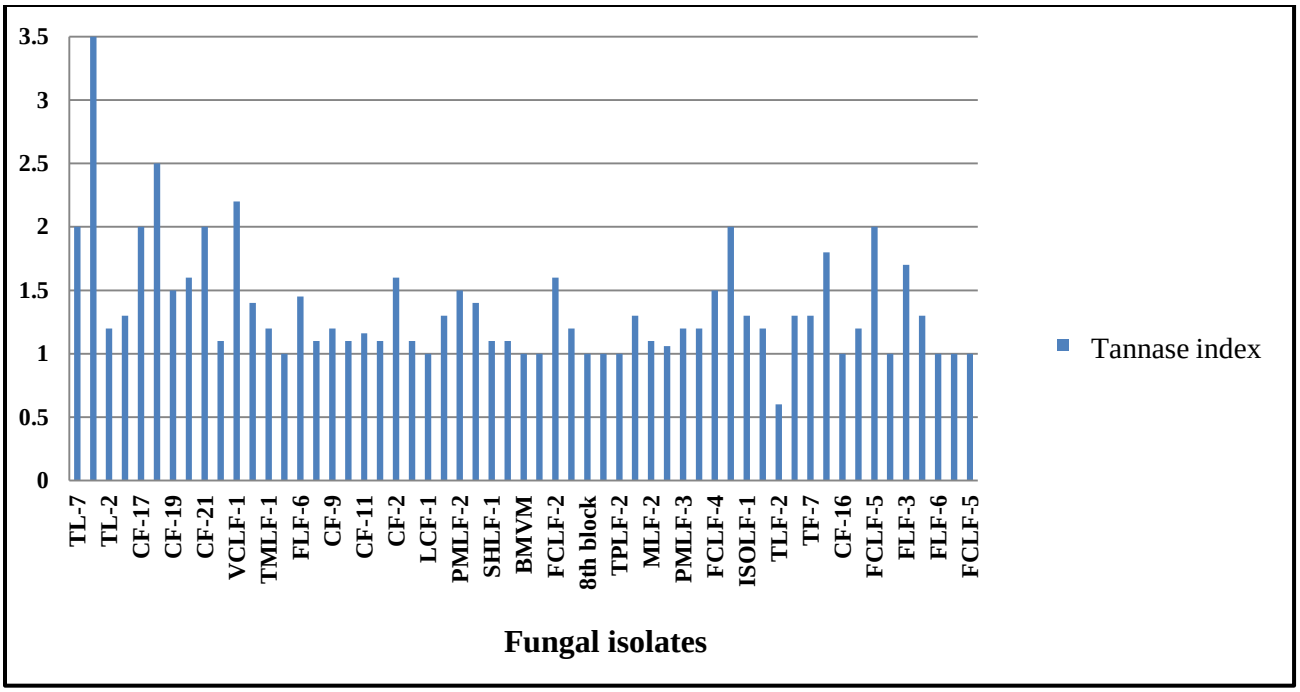
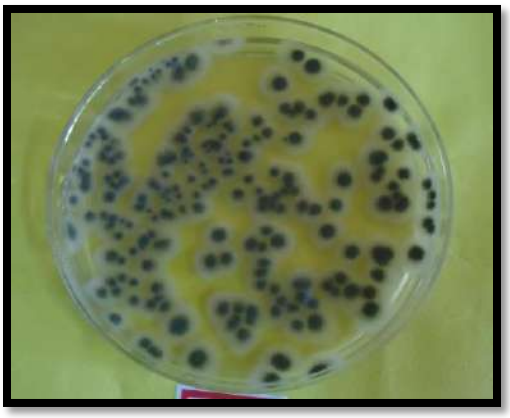


Figure 4.5. Tannase indices of elite fungal isolates

Table 4.4. Tannase indices of elite fungal isolates

Name of organism	Tannase index
Aspergillus fumigatus CF-17	2.0
*Penicillium chrysogenum*VCLF-1	2.2
Aspergillus sp. TL-8	3.5

(A)



(B) **(C)**

Plate 4.6. Tannase activity on TAMEA (Tannic Acid Malt Extract Agar)

(A) *Aspergillus sp* TL-8 **(B)** *Penicillium citrinum* CF-20

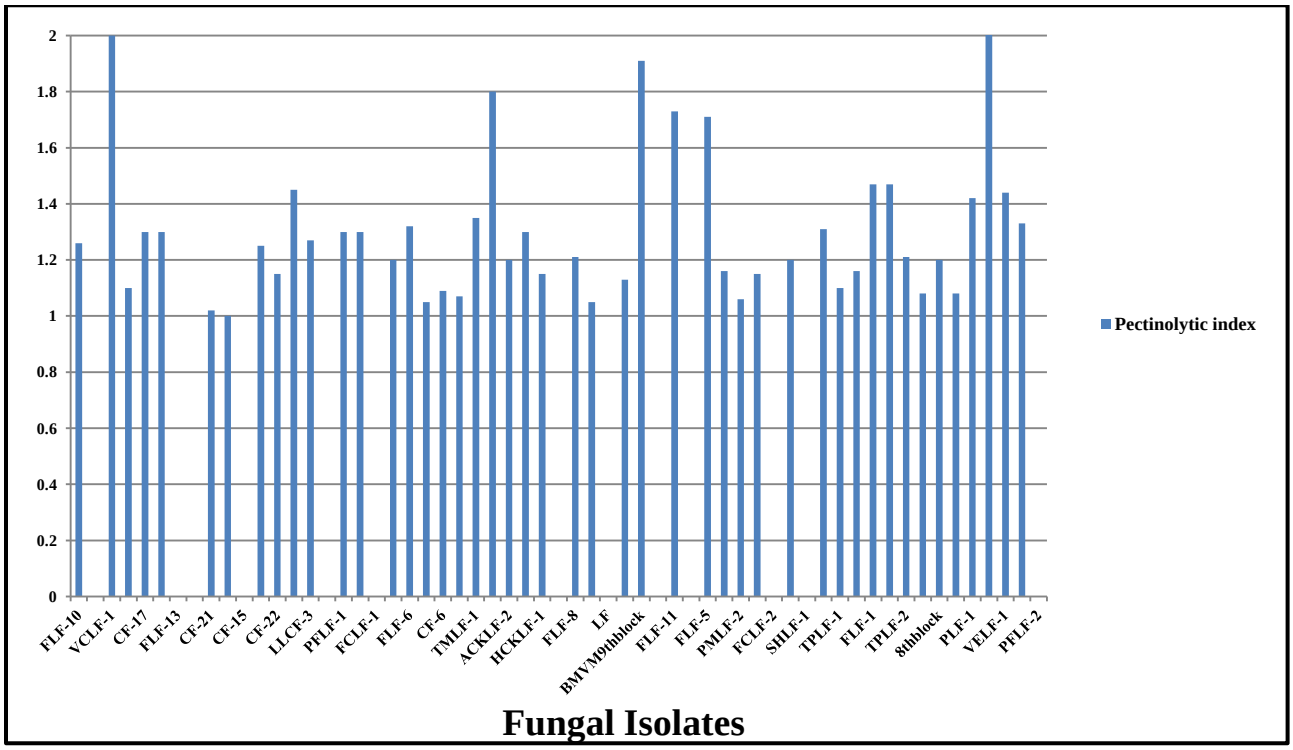
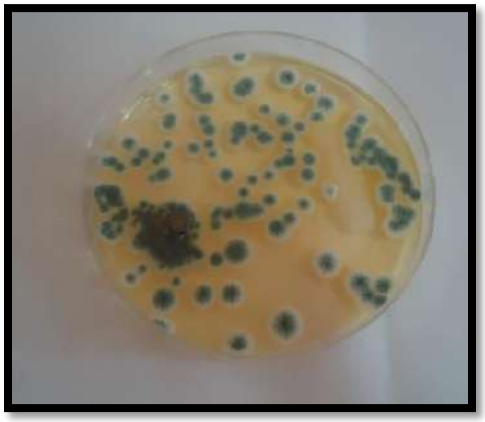


Figure 4.4. Pectinolytic indices of elite fungal isolates

Table 4.5. Pectinolytic indices of elite fungal isolates

Name of organism	Pectinolytic index
Penicillium chrysogenum VCLF-1	2.0
Aspergillus affustus FLF-11	1.7
Alternaria tenuissima LG-1	2.0

(A)



(B) **(C)**

Plate 4.5. (A) Pectinolytic activity on MSA (Mineral Salt Agar with 1%pectin), **(B)** Elite pectinolytic fungal isolates *Alternaria tenuissima* LG-1 (C) *Penicillium chrysogenum* VCLF-1

4.2.6. Qualitative screening of fungi for lignolytic activity

4.2.6.1. Bavendamm test for total polyphenol oxidase activity

Out of the seventy fungal isolates, ten fungal isolates *viz., Purpureocillium lilacinum* FLF-6, *Trichoderma hamatum* FLF-13, *Ceratocystis paradoxsa* CP-1, *Xylaria sp*. TF-4, *Aspergillus fumigatus* CF-17, *Penicillium chrysogenum* VCLF-1, *Curvularia aeria* TL-8, *Alternaria alternata* FLF-10, *Neopestalotiopsis australis* FLF-2 and *Alternaria tenuissima* LG-1 were positive for total polyphenol oxidase activity on Tannic Acid Malt Extract Agar (Plate 4.7 and Table 4.6).

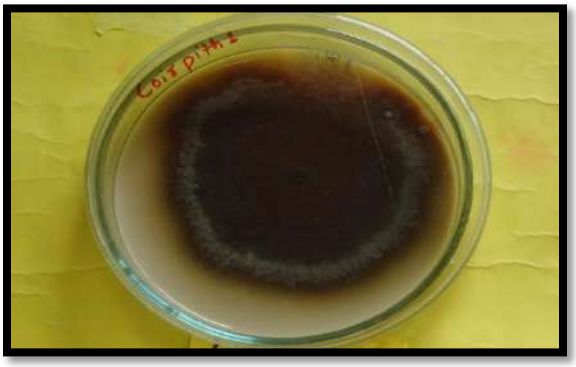
4.2.6.2. Qualitative screening of fungi for laccase, lignin and manganese peroxidase activities

Out of seventy fungal isolates, ten fungal isolates were positive for laccase activity (Plate 4.7). But none of the isolates were positive for peroxidase activity (Table 4.6). The elite lignolytic fungal isolates *viz*., *Purpureocillium lilacinum* FLF-6, *Trichoderma hamatum* FLF-13 and *Xylaria* sp.TF-4 are presented in (Plate 4.8).

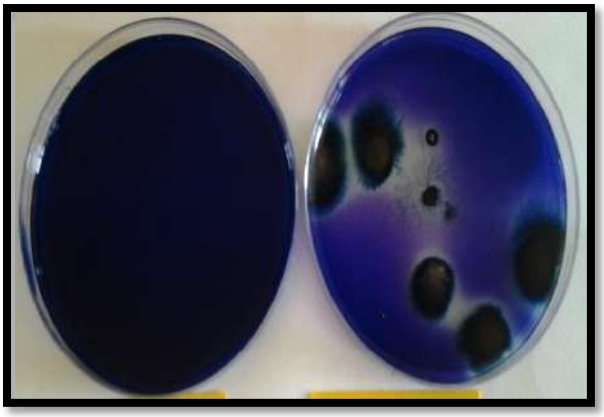
Table 4.6. Polyphenol oxidase, laccase and peroxidase activities of elite fungal isolates

Name of fungal isolate	Polyphenol oxidase activity	Laccase activity	Peroxidase activity
Purpureocillium lilacinum FLF-6	+	+	-
Trichoderma hamatum FLF-13	+	+	-
Ceratocystis paradoxa CP-1	+	+	_
Xylaria sp TF-4	+	+	_
Aspergillus fumigatus CF-17	+	+	_
Penicillium chrysogenum VCLF-1	+	+	_
*Aspergillus sp.*TL-8	+	+	_
Alternaria alternata FLF-10	+	+	_
Neopestalotiopsis australis FLF-2	+	+	_
Alternaria tenuissima LG-1	+	+	_

(+ Positive; - negative)



(A)



(B) **(C)**

Plate 4.7. Lignolytic activity of fungal isolates **(A)** Total polyphenol oxidase activity on TAMEA **(B)** Laccase activity on ABTS agar **(C)** Peroxidase activity on Azure-B agar

(A)

(B)

(C)

Plate 4.8. Elite lignolytic fungi (A) *Purpureocillium lilacinum* FLF-6 (B) *Trichoderma hamatum* FLF-13 (C) *Xylaria* sp. TF-4

4.3. Molecular identification of elite isolates

The elite bacterial and fungal isolates were identified by molecular characterization as *Bacillus cereus* CB-7, *Bacillus* sp. CB-9, *Bacillus invictae* CB-16, *Bacillus tequilensis* CB-17, *Bacillus simplex* FLCB-5 and *Bacillus endophyticus* FLCB-11. The elite fungal isolates were identified as *Penicillium citrinum* CF-20, *Aspergillus* sp. TMLF-1, *Aspergillus niger* PMLF-1, *Aspergillus* sp. FCLF-1, *Aspergillus* sp. CF-11, *Penicillium chrysogenum* VCLF-1, *Aspergillus oryzae* CP-2, *Aspergillus* sp. CF-6, *Aspergillus fumigatus* CF-17, *Aspergillus* sp. TL-8, *Aspergillus affustus* FLF-11, *Alternaria tenuissima* LG-1, *Purpureocilium lilacinum* FLF-6, *Trichoderma hamatum* FLF-13, *Ceratocystis paradoxa* CP-1, *Xylaria* sp. TF-4, *Aspergillus* sp. TL-8, *Alternaria alternata* FLF-10 and *Neopestalotiopsis australia* FLF-2.

4.4. Enzyme assays

4.4.1. Total Cellulase activity

Among bacterial isolates *Bacillus tequilensis* CB-17 recorded the highest total cellulase activity of 0.0169 IU/ml followed by *Bacillus cereus* CB-7 of 0.016 IU/ml (Table 4.7) (Figure 4.6). Among the fungal isolates *Aspergillus sp.*FCLF-1 recorded the highest total cellulase activity of 0.049 IU/ml followed by *Aspergillus sp.*TMLF-1 and *Penicillium citrinum* CF-20 (Table 4.8) (Figure 4.7).

4.4.2. Xylanase activity

The isolate *Aspergillus sp.*CF-11 showed the highest xylanase activity of 0.09 µmole/ml/min followed by *Aspergillus oryzae* CP-2 (Table 4.9) (Figure 4.8).

Table 4.7. Total cellulase activity of elite bacterial isolates (FPA method)

Cellulolytic bacteria	**Total cellulase activity (IU/ml)**
Bacillus cereus CB-7	0.016
Bacillus sp. CB-9	0.008
Bacillus invictae CB-16	0.012
Bacillus tequilensis CB-17	0.016
Bacillus simplex FLCB-5	0.008
Bacillus endophyticus FLCB-11	0.004

Table 4.8. Total cellulase activity of elite fungal isolates (FPA method)

Name of isolate	**Total cellulase activity (IU/ml)**
Penicillium citrinum CF-20	0.022
Aspergillus sp. TMLF-1	0.026
Aspergillus niger PMLF-1	0.012
Aspergillus sp. FCLF-1	0.049

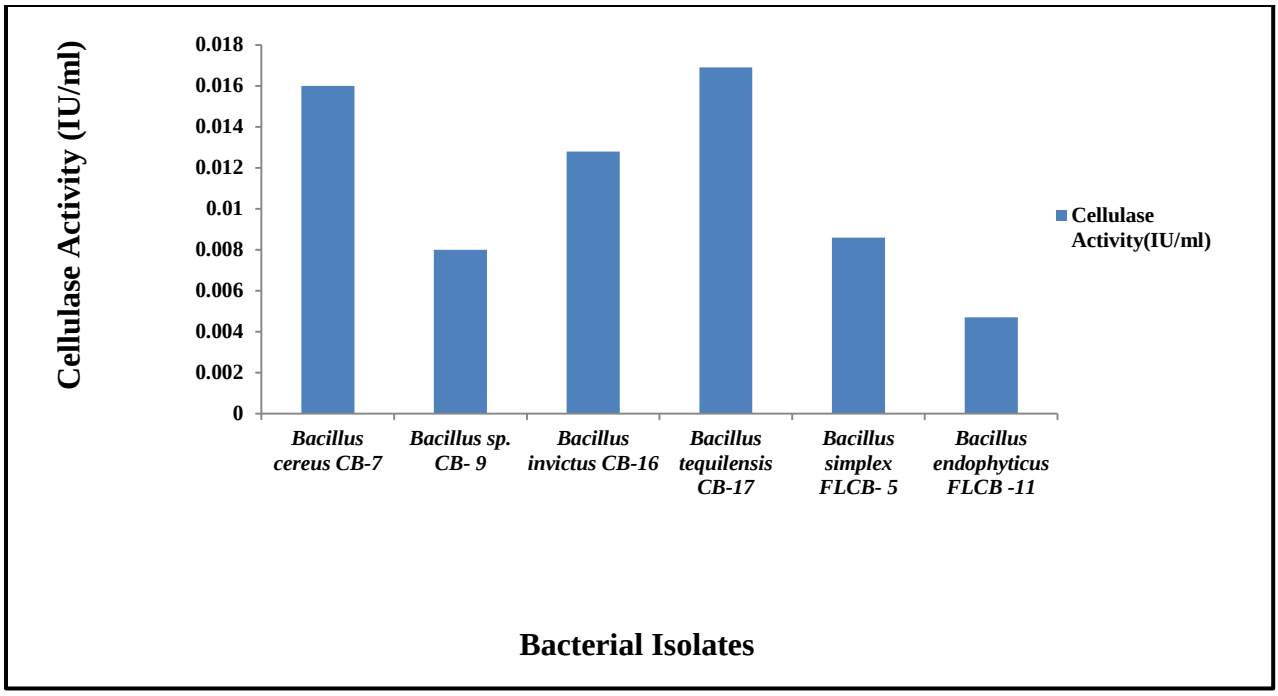


Figure 4.6. Total cellulase activity of the elite bacterial isolates

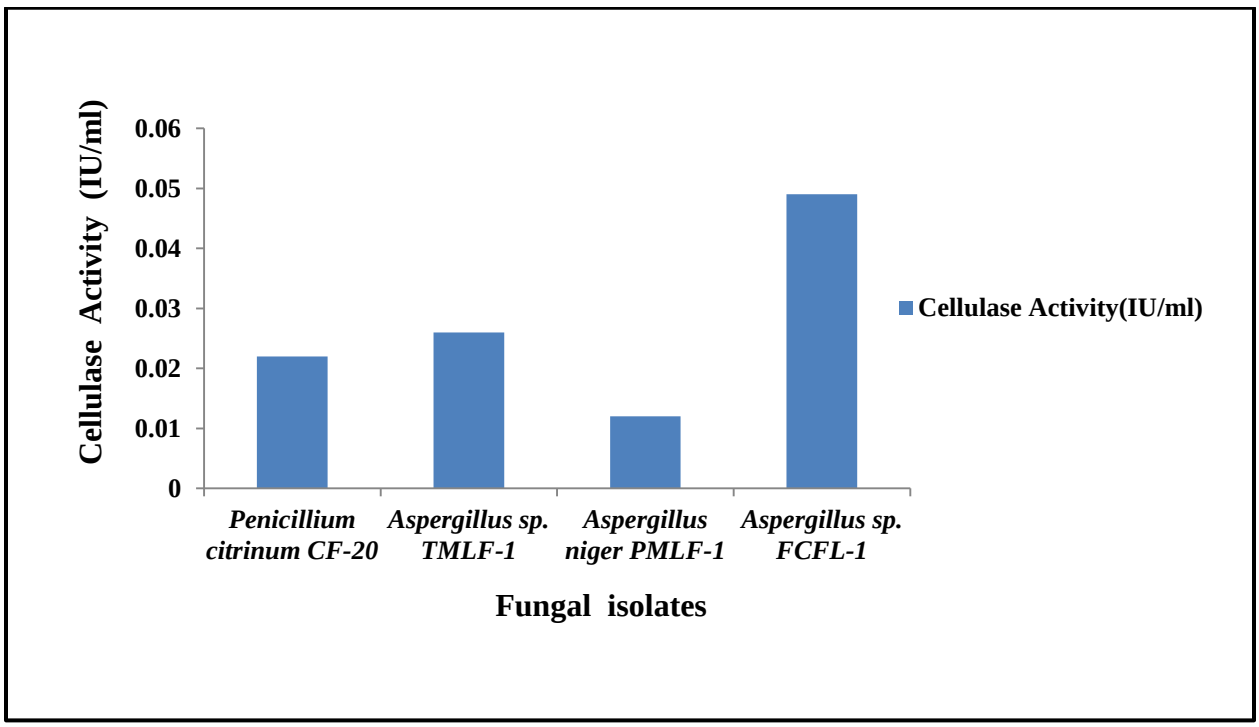


Figure 4.7. Total cellulase activity of elite fungal isolates

4.4.3. Tannase activity

The isolate *Penicillium chrysogenum* VCLF-1 showed the highest tannase activity of 339µg/ml as determined by the HPLC method (Table 4.10) (Figure 4.9).

4.4.4. Pectinase activity

The isolate *Alternaria tenuissima* LG-1 recorded the highest pectinase activity of 2.511 µmole/ml/min followed by *Penicillium chrysogenum* VCLF-1 and *Aspergillus affustus* FLF-11 (Table 4.11) (Figure 4.10).

4.5. *In vitro* screening studies using chilli stalk as substrate

The *in vitro* screening studies yielded partially decomposed chilli stalks after 49 days of incubation. The humification potential ($Q_{4/6}$ ratio) of most elite cellulolytic bacteria, cellulolytic fungi, lignin and tannin degrading fungal isolates were below 5, which indicates the good humification potential of elite isolates (Figure 4.11, 4.12, 4.12A and Plate 4.9).

4.6. Evaluation of compatibility among elite isolates

Based on compatibility test among the elite isolates (Table 4.12 and Plate 4.10) the cellulolytic bacterium *Bacillus cereus* CB-7 was found to be more compatible with the cellulolytic bacterium *Bacillus endophyticus* FLCB-11, tannin degrading fungus *Aspergillus sp*. TL-8 and lignolytic fungus *Trichoderma hamatum* FLF-13. The cellulolytic bacterium *Bacillus endophyticus* FLCB-11 was found to be more compatible with the tannin degrading fungus. *Aspergillus sp.* TL-8, tannin degrading fungus *Penicillium chrysogenum* VCLF-1 and lignolytic fungus *Trichoderma hamatum* FLF-13. The cellulolytic fungus *Penicillium citrinum* CF-20 was found to be more compatible with the tannin degrading fungus *Aspergillus sp*.TL-8, tannin

degrading fungus *Penicillium chrysogenum* VCLF-1, lignolytic fungus *Trichoderma hamatum* FLF-13, lignolytic fungus *Aspergillus affustus* FLF-11 and xylanolytic fungus *Alternaria tenuissima* LG-1. The cellulolytic fungus *Aspergillus sp.* TMLF-1 was found to be more compatible with the cellulolytic bacterium *Bacillus endophyticus* FLCB-11, cellulolytic fungus *Penicillium citrinum* CF-20, tannin degrading *Aspergillus sp.* TL-8, xylanolytic fungus *Aspergillus oryzae* CP-2 and the lignolytic fungus *Aspergillus sp.*FLF-13. The tannin degrading *Aspergillus sp.* TL-8 was found to be more compatible with cellulolytic bacterium *Bacillus cereus* CB-7, cellulolytic bacterium *Bacillus endophyticus* FLCB-11, cellulolytic fungus *Aspergillus sp.*TMLF-1, tannin degrading fungus *Penicillium chrysogenum* VCLF-1 and xylanolytic fungus *Ceratocystis paradoxa* CP-1. The xylanolytic fungus *Aspergillus oryzae* CP-2 was found to be more compatible with cellulolytic fungus *Aspergillus sp.* TMLF-1 and lignolytic fungus *Aspergillus sp.* FLF-13. The lignolytic fungus *Aspergillus affustus* FLF-11 was found to be more compatible with cellulolytic fungus *Penicillium citrinum* CF-20 and lignolytic fungus *Trichoderma hamatum* FLF-13. The lignolytic fungus *Trichoderma hamatum* FLF-13 was found to be more compatible with cellulolytic bacterium *Bacillus cereus* CB-7, cellulolytic bacterium *Bacillus endophyticus* FLCB-11, cellulolytic fungus *Penicillium citrinum* CF-20, cellulolytic fungus *Aspergillus sp.*TMLF-1, tannin degrading fungus *Aspergillus sp.* TL-8, tannin degrading fungus *Penicillium chrysogenum* VCLF-1 and the lignolytic fungus *Aspergillus affustus* FLF-11.

Table 4.9. Xylanase activity of elite fungal isolates

Name of isolate	Xylanase activity (µ mole/ml/min)
Aspergillus sp. CF-11	0.090
Penicillium chrysogenum VCLF-1	0.018
Aspergillus oryzae CP-2	0.044
Aspergillus sp. CF-6	0.019

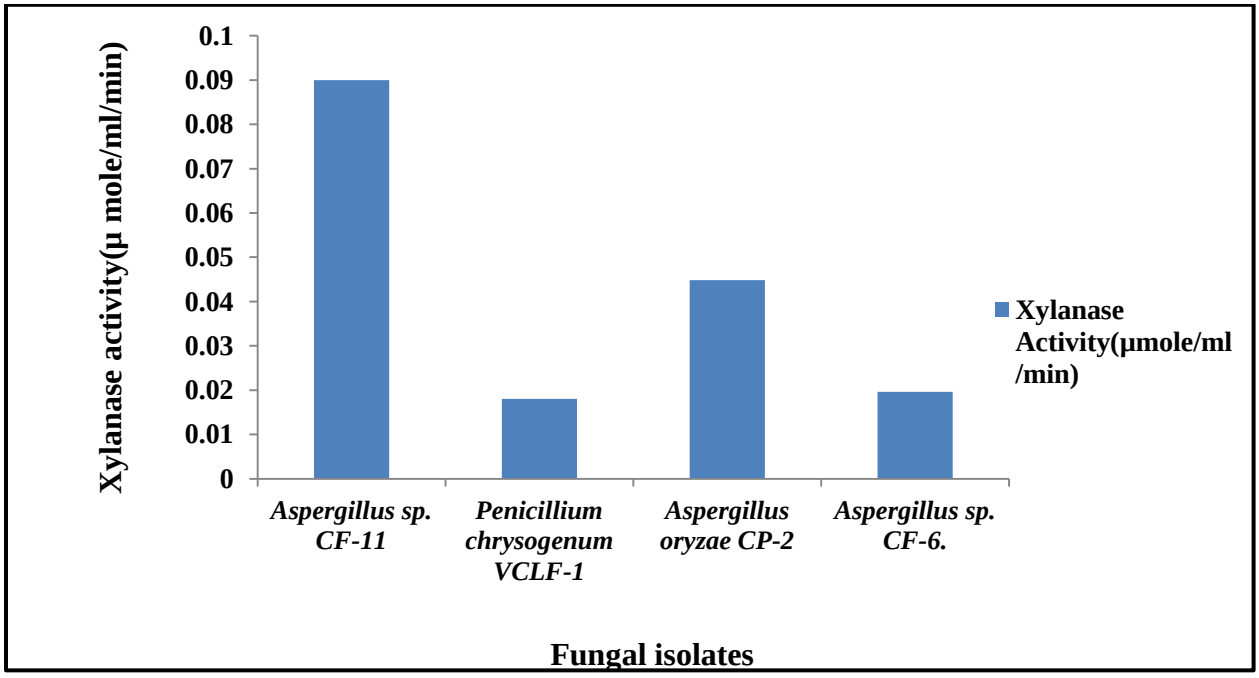


Figure 4.8. Xylanase activity of elite fungal isolates

Table 4.10. Tannase activity of elite fungal isolates determined by HPLC

Name of isolate	Concentration of gallic acid (µg/ml)
Aspergillus fumigatus CF-17	254
Penicillium chrysogenum VCLF-1	339
Aspergillus sp. TL-8	270

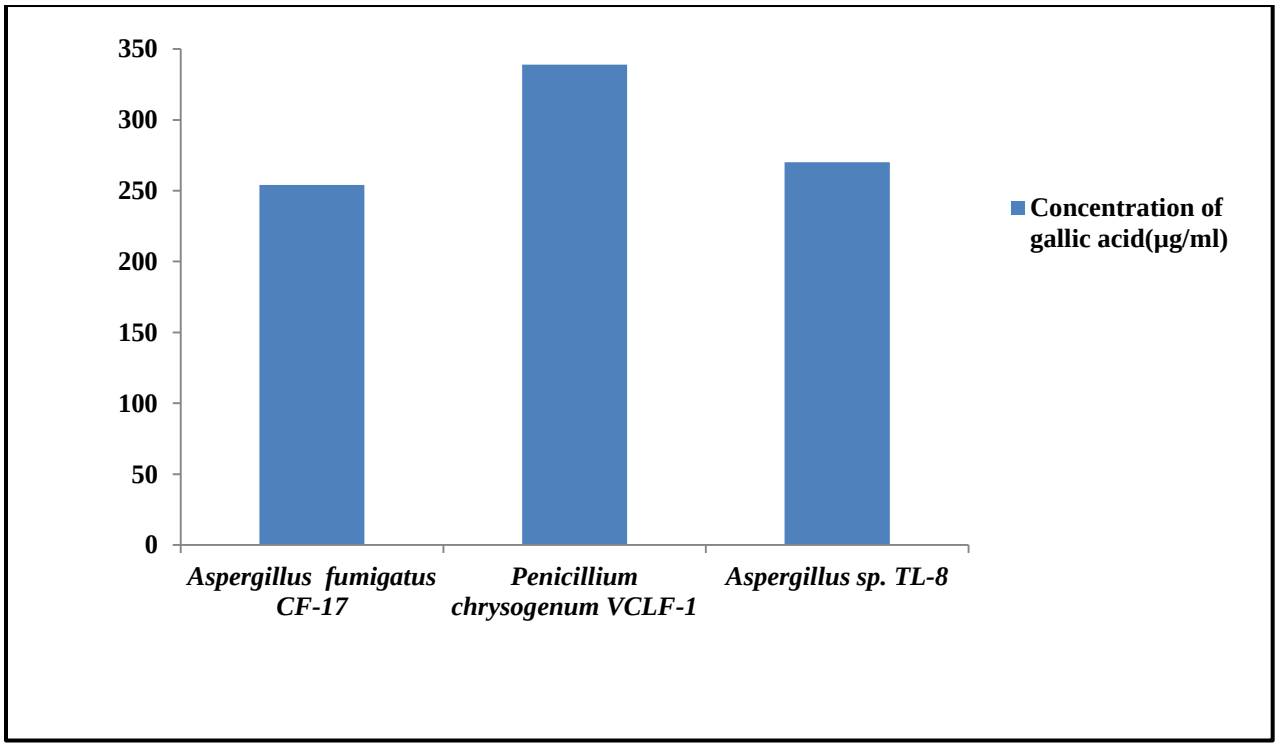


Figure 4.9. Tannase activity of elite fungal isolates

Table 4.11. Pectinase activity of elite fungal isolates

Name of isolate	Pectinase activity (µ mole/ml/min)
*Penicillium chrysogenum*VCLF-1	1.776
Aspergillus affustus FLF-11	1.193
Alternaria tenuissima LG-1	2.511

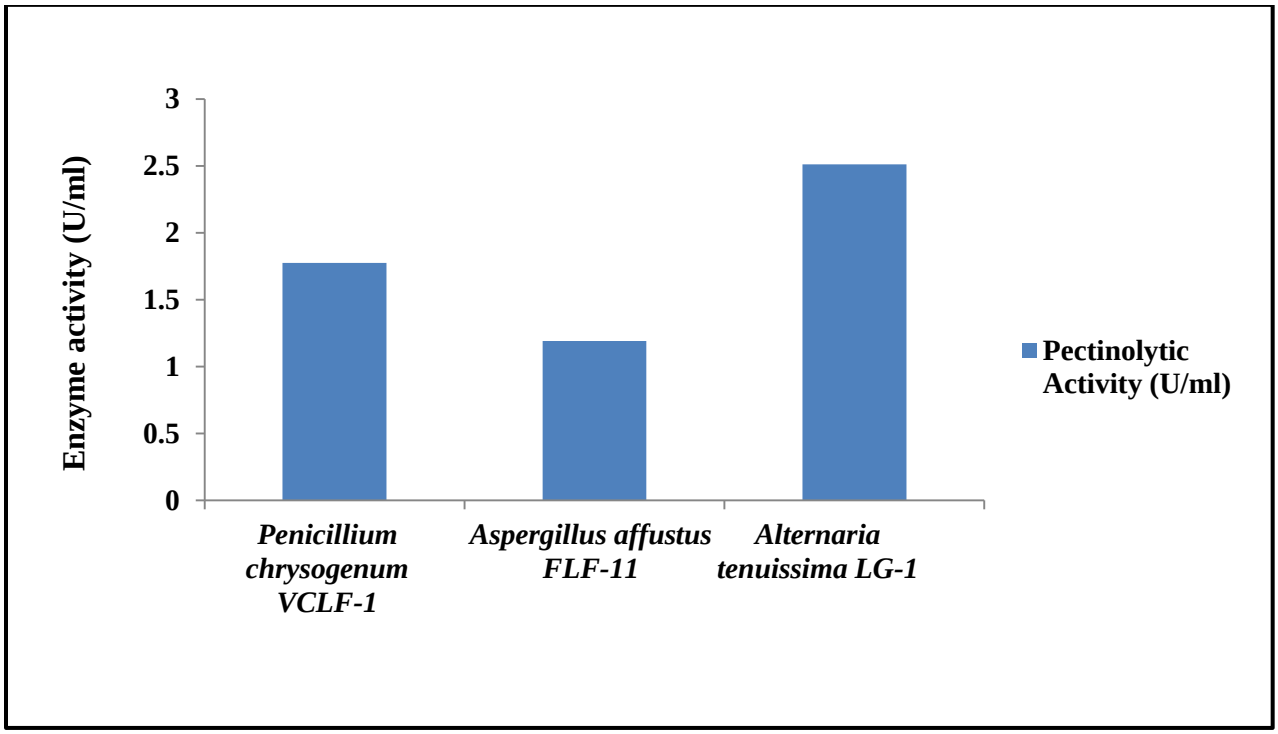


Figure 4.10. Pectinase activity of elite fungal isolates

(A) **(B)**

Plate 4.9. *In vitro* screening studies using chilli stalk as substrate **(A)** Initial substrate

(B) Partially decomposed substrate (42 days)

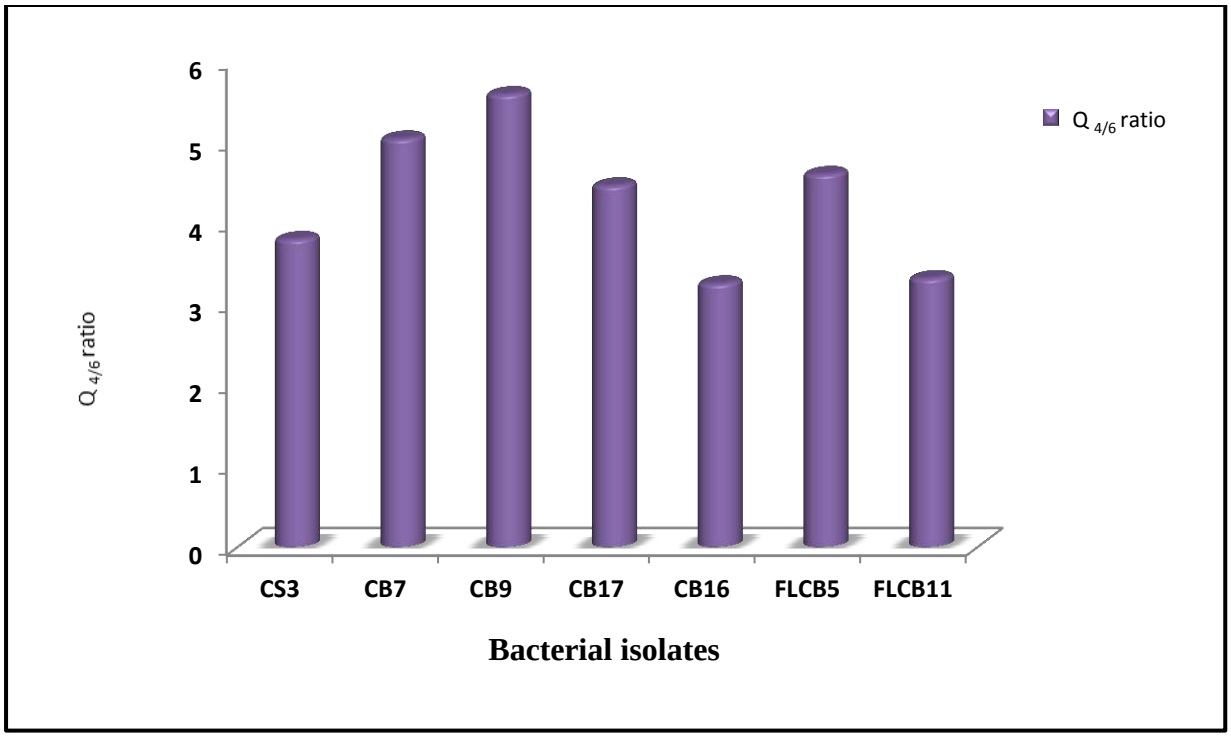


Figure 4.11. Humification potential of elite cellulolytic bacterial isolates

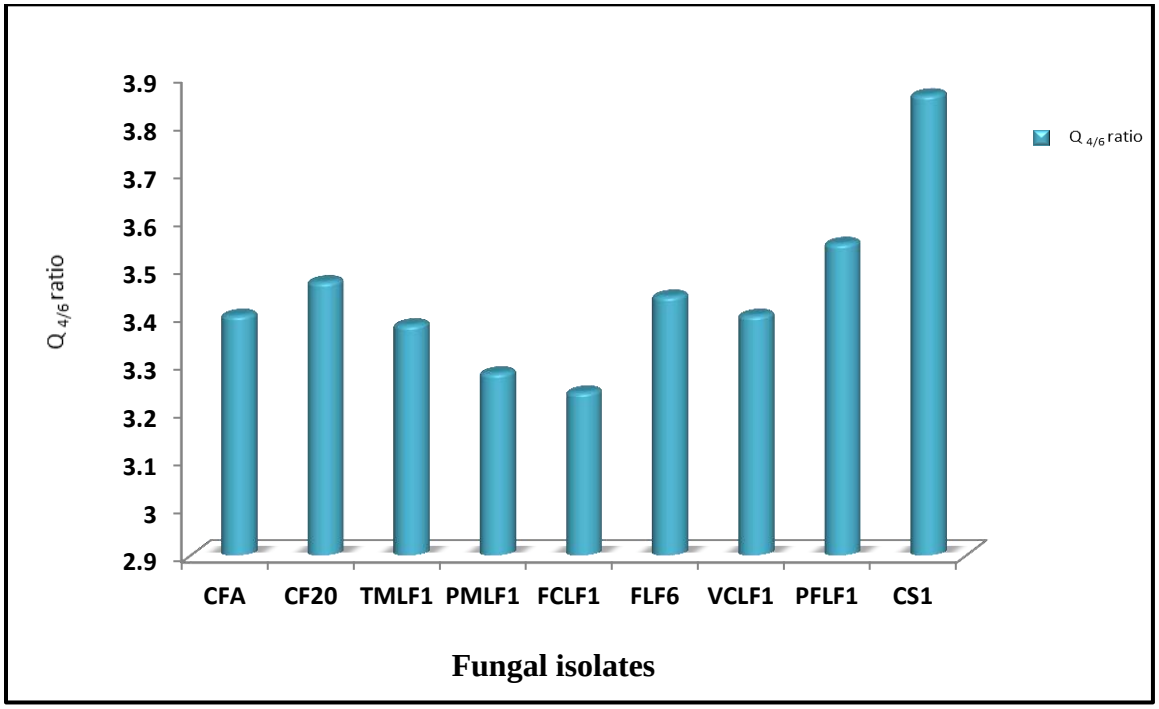


Figure 4.12. Humification potential of elite cellulolytic fungal isolates

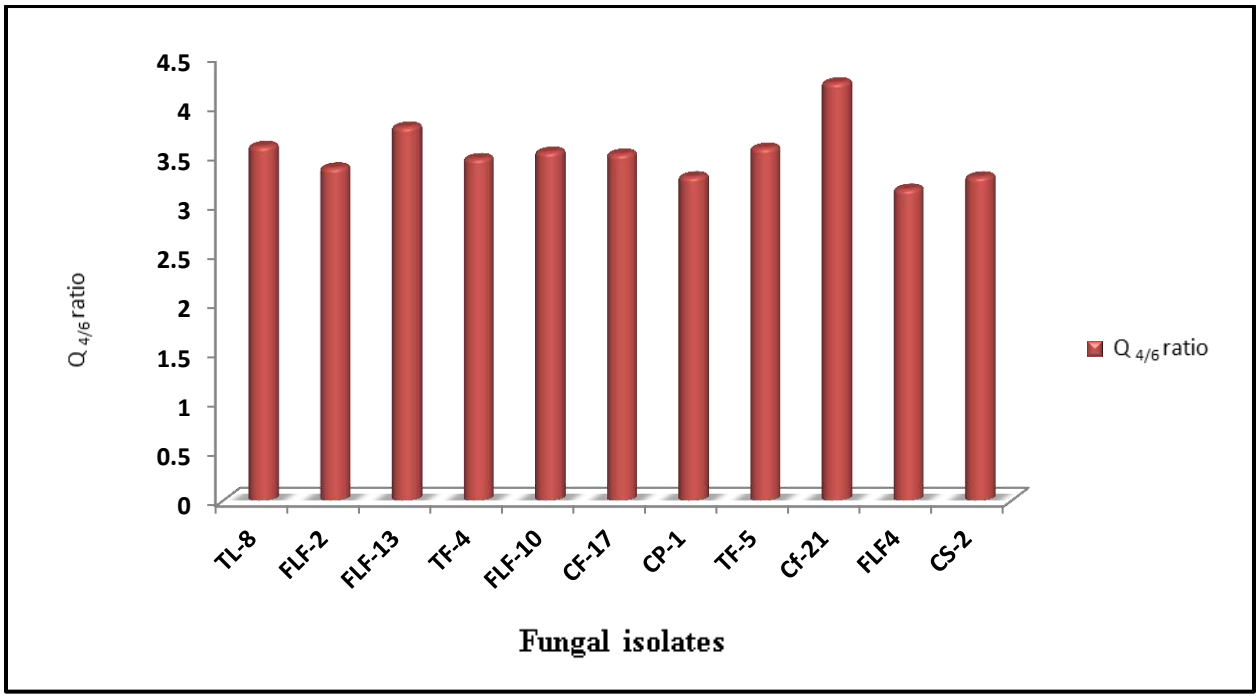


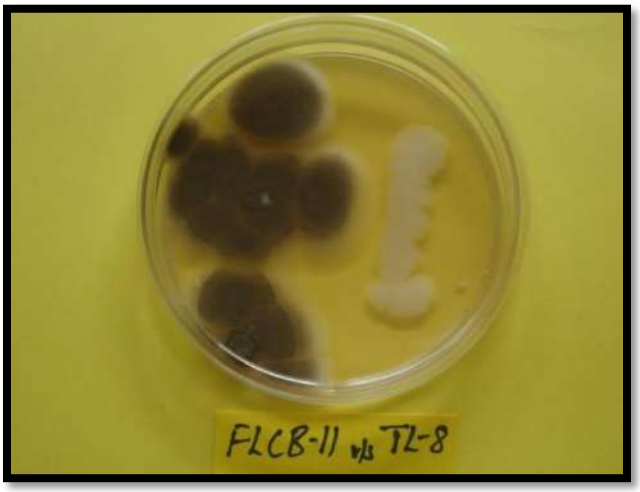
Figure 4.12A. Humification potential of elite lignolytic and tannin degrading fungal isolates

Table 4.12. Compatibility amongst the elite isolates

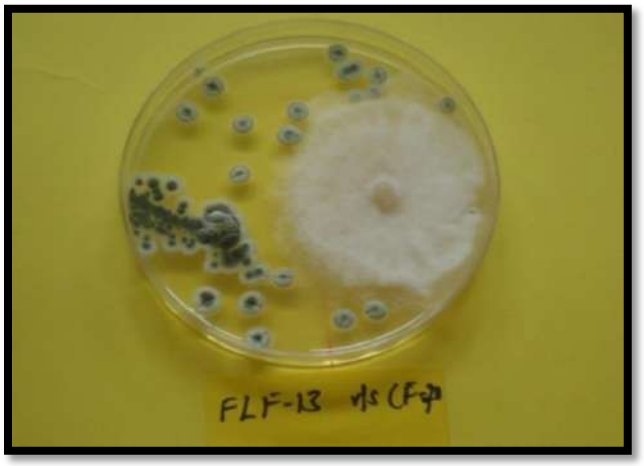
Test Organism	Cellulolytic Bacteria	Cellulolytic Fungi	Tannin Degrading Fungi	Hemicellulolytic Fungi	Pectinolytic Fungi	Lignolytic Fungi						
	CB-7	FLCB-11	CF-20	TMLF-1	TL-8	VCLF-1	CP-1	CP-2	FLF-11	LG-1	FLF-10	FLF-13
CB-7	NA	+	+	+	++	+	-	+	+	+	++	++
FLCB-11	NA	NA	+	+	++	++	++	-	+	+	+	++
CF-20	+	+	NA	++	++	++	-	+	++	++	+	++
TMLF-1	+	++	++	NA	++	+	-	++	+	+	+	++
TL-8	++	++	++	++	NA	++	++	+	+	+	+	+
VCLF-1	+	+	++	++	+	NA	+	+	+	+	+	+
CP-1	-	++	+	-	+	+	NA	++	+	+	-	+
CP-2	+	-	+	++	+	+	++	NA	+	+	+	++
FLF-11	+	+	++	+	+	+	+	+	NA	+	+	++
LG-1	+	+	++	++	+	+	+	+	+	NA	++	++
FLF-10	++	+	+	+	++	+	-	+	+	++	NA	+
FLF-13	++	++	++	++	++	++	-	+	++	+	+	NA

+ compatible; ++ highly compatible; - non compatible; NA-not applicable

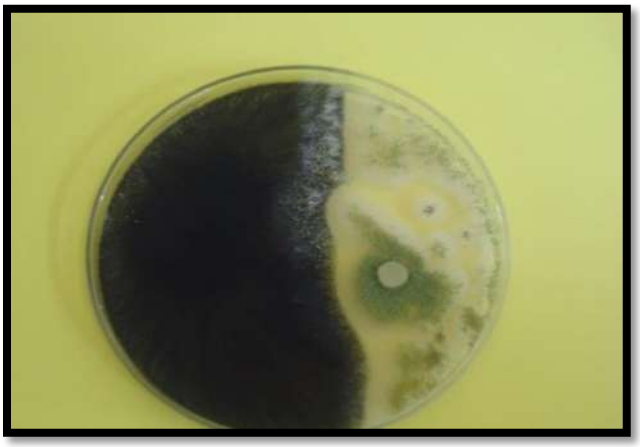
(A) *Bacillus cereus* CB-7 vs *Bacillus endophyticus* FLCB-11



(B) *Bacillus endophyticus* FLCB-11 vs *Aspergillus* sp. TL-8



(C) *Trichoderma hamatum* FLF-13 vs *Penicillium citrinum* CF-20



(D) *Ceratocystis paradoxa* CP-1 vs *Aspergillus* sp. TMLF-1

Plate 4.10. Compatibility test among selected isolates

4.7. Compost trials using different horticultural residues

During composting the parameters such as pH, Electrical Conductivity (EC), Total Organic Matter (TOM), Total Organic Carbon (TOC), Humification Index (HI) and moisture content were monitored at periodic intervals.

The pH of mango leaf litter compost gradually decreased during initial days of composting and increased subsequently as composting progressed (Figure 4.13). The Electrical Conductivity (EC) increased gradually over the period of composting (Figure 4.14). Total Organic Matter (TOM) increased (Figure 4.16) and Total Organic Carbon (TOC) decreased (Figure 4.17) during composting. The Humification Index (HI) decreased from 7 to 4.1 (Figure 4.18) and the moisture content was around 30-40% in the final compost (Figure 4.15 and Plate 4.11 A)

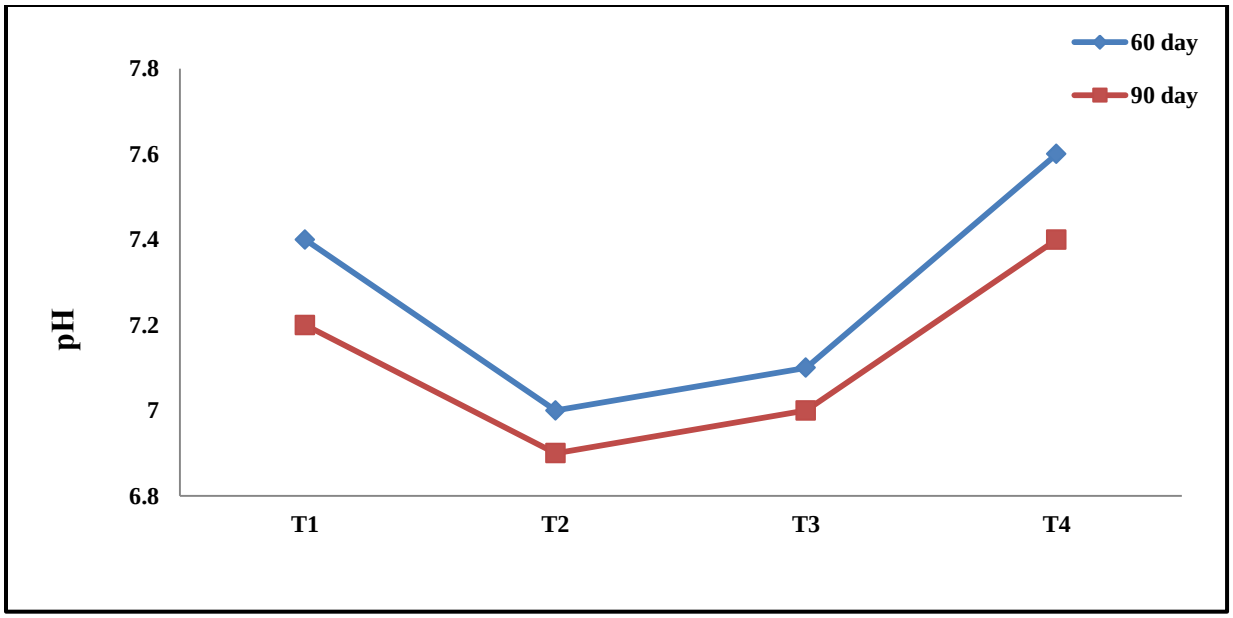
The pH of *Dolichos* stover compost was high initially and decreased gradually as the composting progressed (Figure 4.19). The EC increased gradually over the period of composting (Figure 4.20). The TOM increased (Figure 4.22) and TOC decreased (Figure 4.23) during composting. The HI decreased from 5.2 to 4.2 (Figure 4.24) and the moisture content was around 30-40% in the final compost (Figure 4.21 and Plate 4.11 B).

The pH of chilli stalks compost was high initially (8.4) and decreased (7.2) gradually as the composting progressed (Figure 4.25). The EC decreased gradually over the period of composting (Figure 4.26). The TOM (Figure 4.28) and TOC decreased (Figure 4.29) during composting. The HI decreased from 7.4 to 6.0 (Figure 4.30) and the moisture content was around 30-40% in the final compost (Figure 4.27 and Plate 4.12 A).

The pH of grape pruning's compost was high (8.0) initially and decreased subsequently as composting progressed (Figure 4.31). The EC increased gradually over the period of composting (Figure 4.32), the TOM increased (Figure 4.34) and TOC decreased (Figure 4.35) during composting. The HI decreased from 8 to 5.2 (Figure 4.36) and the moisture content was around 30-40% in the final compost (Figure 4.33 and Plate 4.12 B).

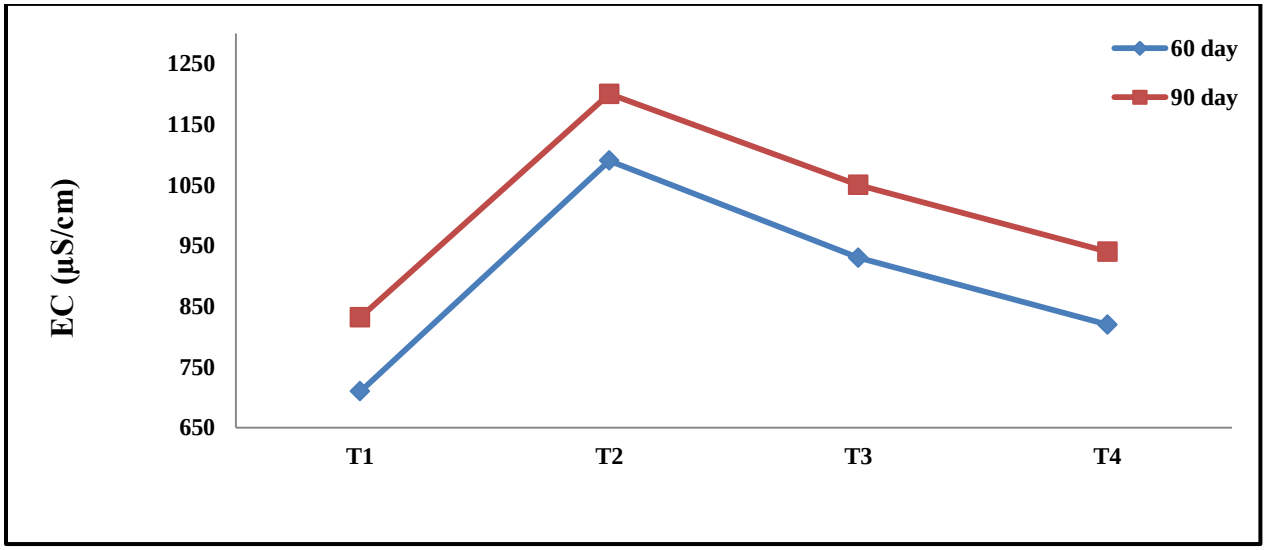
The pH of brinjal stalks compost was high (8.1) initially and decreased (7.0) subsequently as composting progressed (Figure 4.37). The EC increased gradually over the period of composting (Figure 4.38). The TOM increased (Figure 4.40) and TOC decreased (Figure 4.41) during composting. The HI decreased from 8 to 4.5 (Figure 4.42) and the moisture content was around 30-40% in the final compost (Figure 4.39 and Plate 4.13 A).

Mango leaf litter and *Dolichos* stover were converted to good quality compost in a 90-day period, while chilli stalks, brinjal stalks and grape pruning's were converted to stable and mature composts in 150 days.



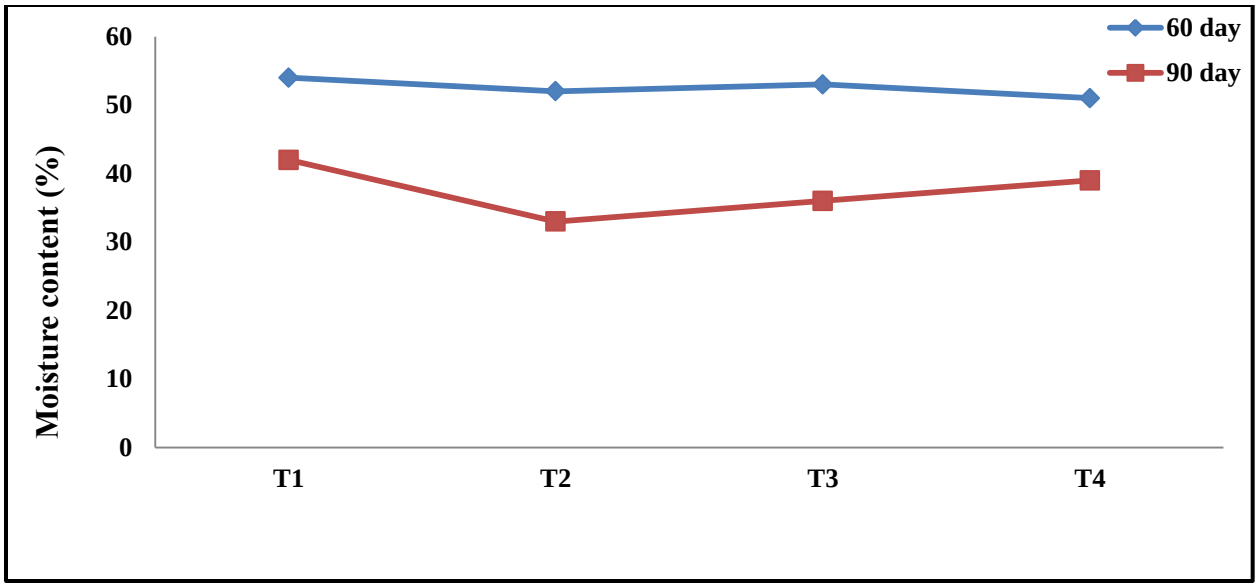
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.13. Progression of pH in mango leaf litter compost over time



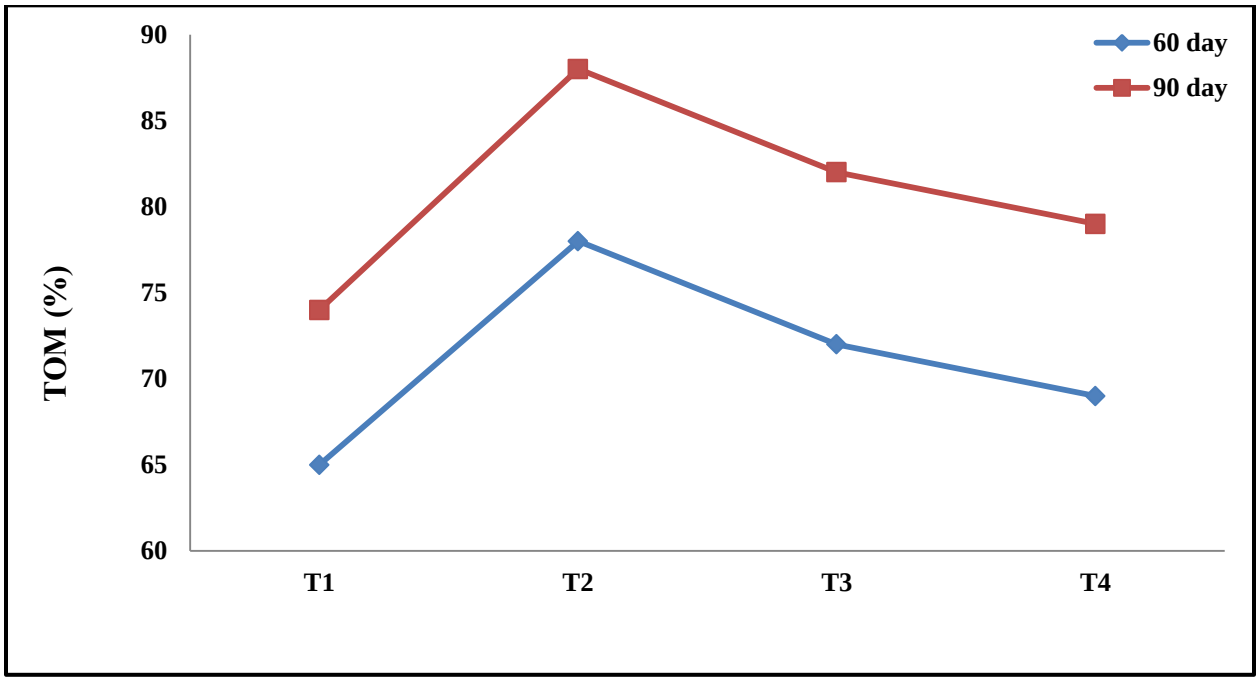
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.14. Progression of Electrical Conductivity (EC) in mango leaf litter compost over time



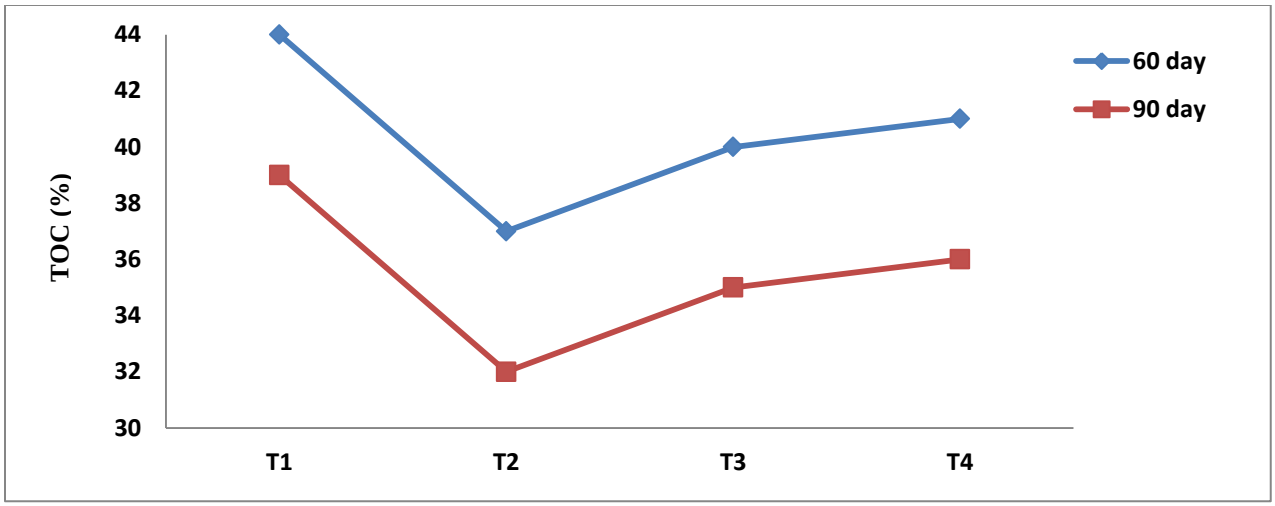
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.15. Progression of moisture content in mango leaf litter compost over time



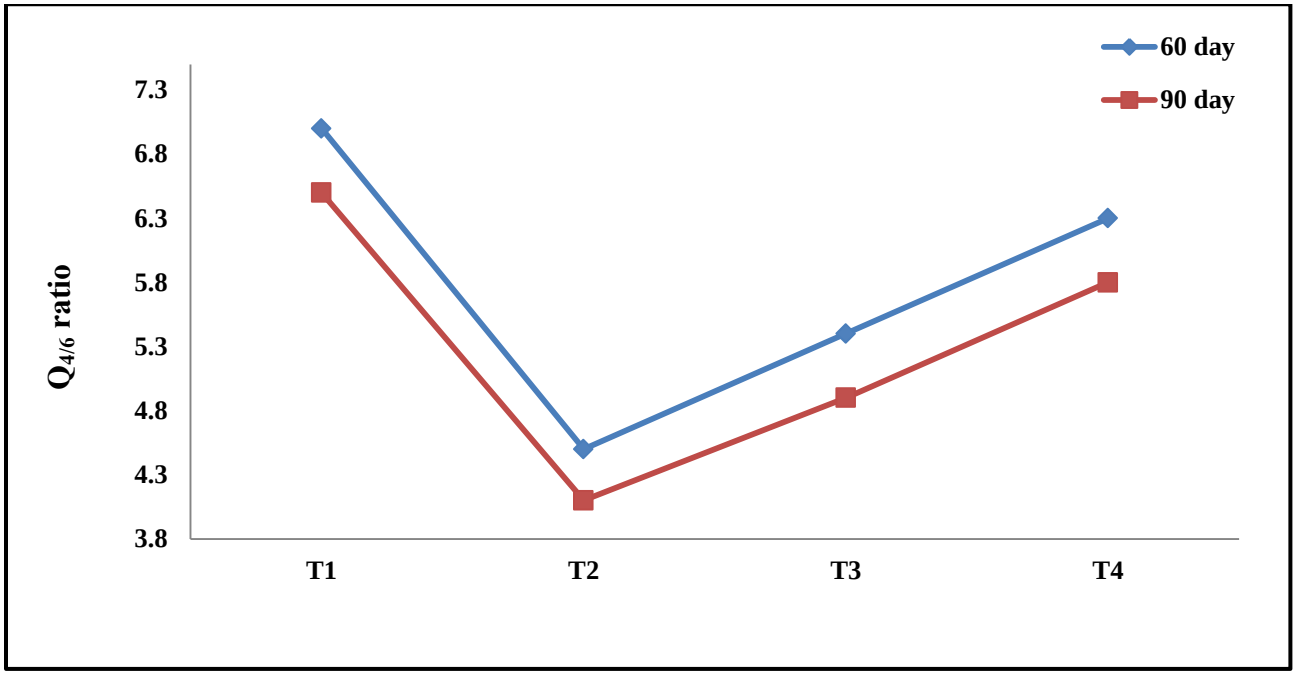
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.16. Progression of Total Organic Matter (TOM) in mango leaf litter compost over time



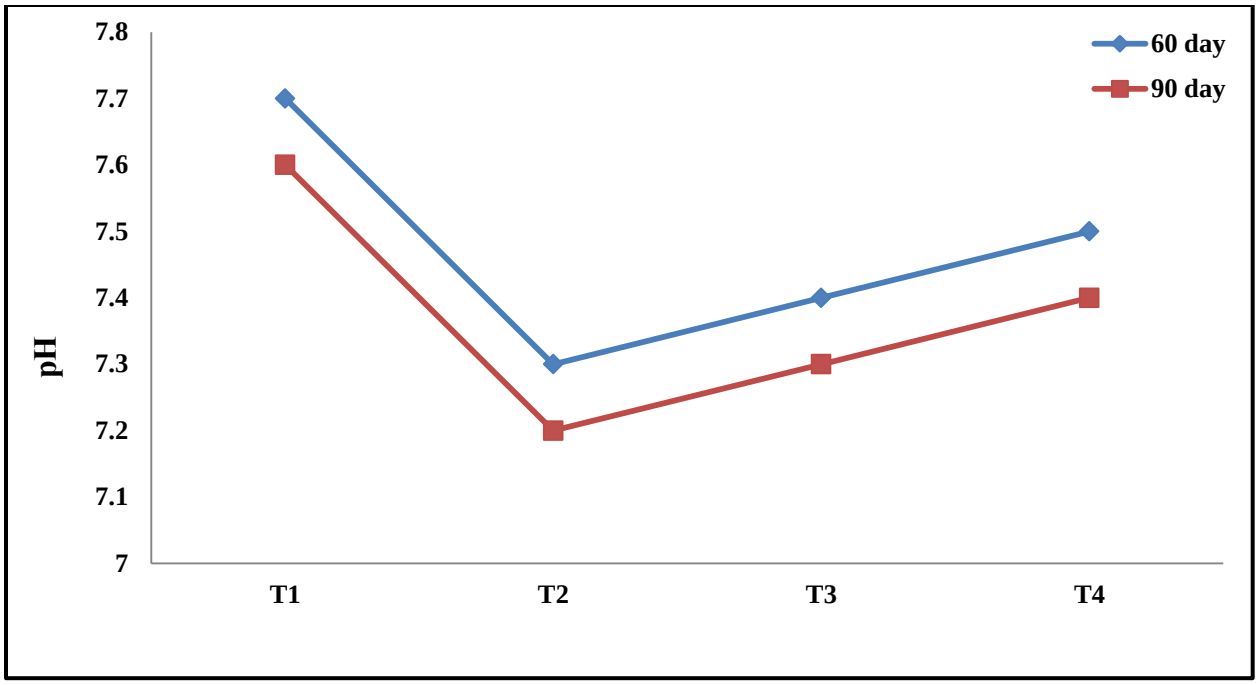
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.17. Progression of Total Organic Carbon (TOC) in mango leaf litter compost over time



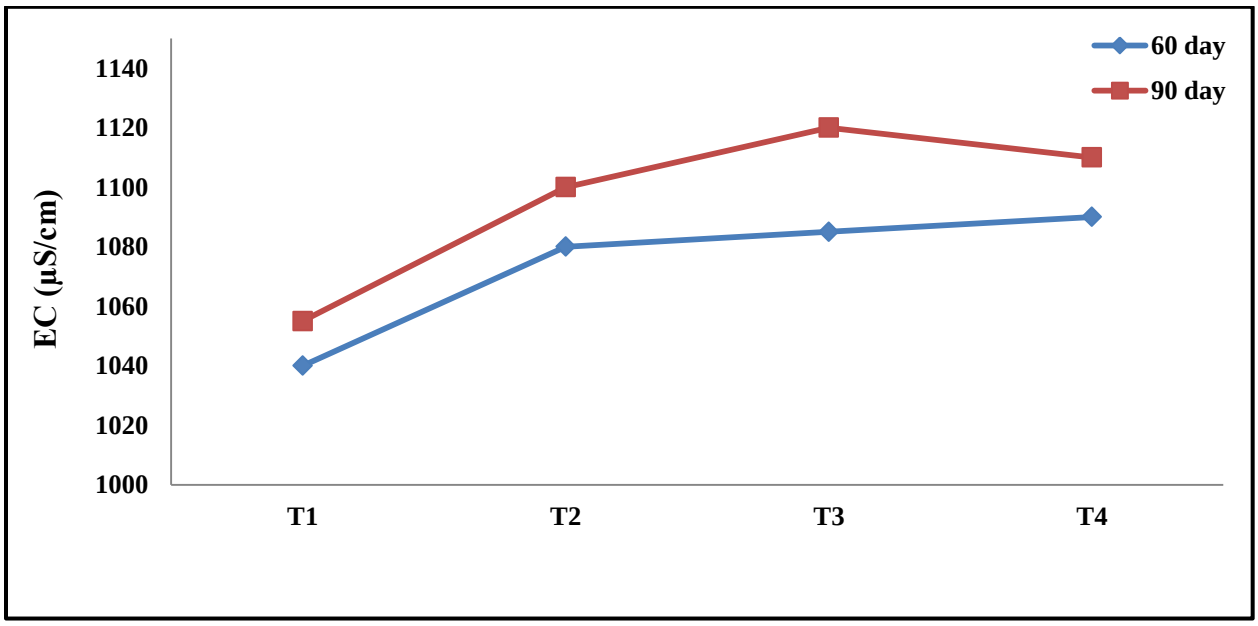
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.18. Progression of Humification Index (HI) in mango leaf litter compost over time



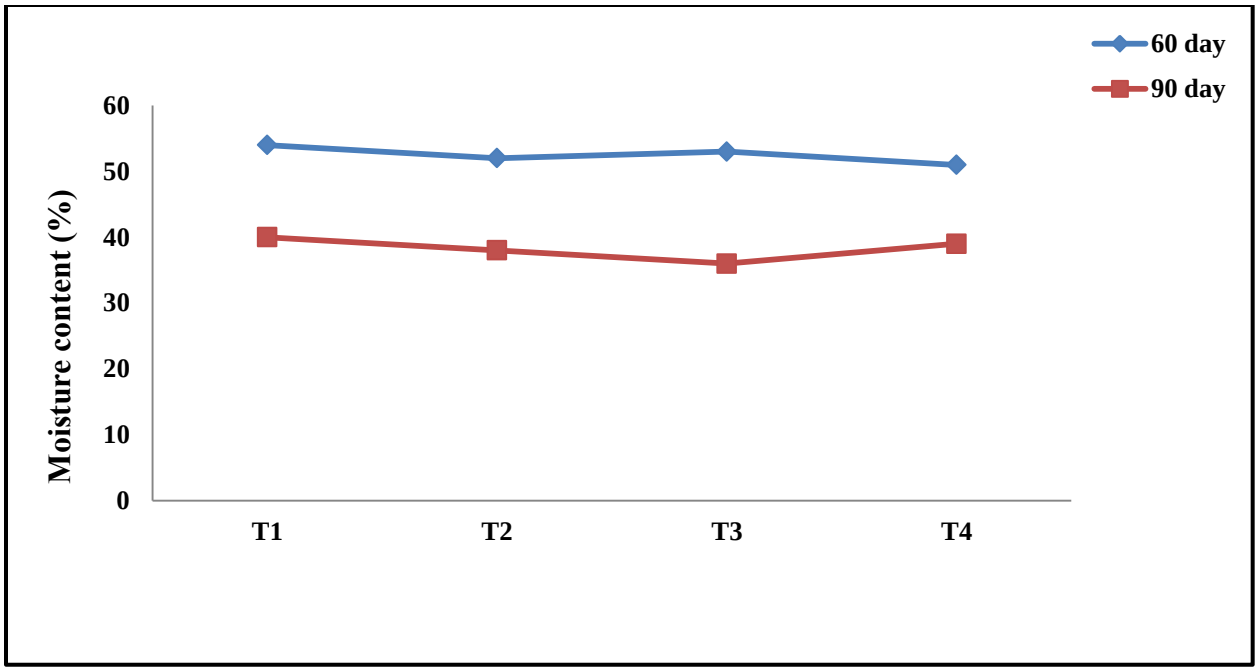
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.19. Progression of pH in *Dolichos* stover compost over time



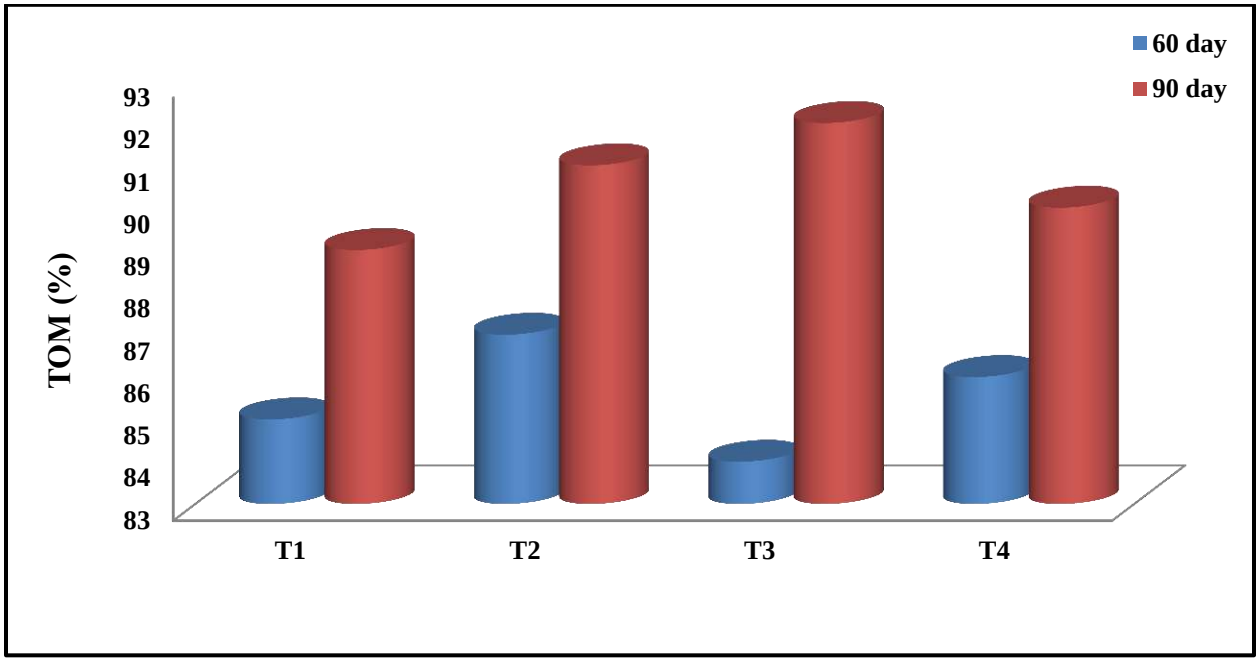
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.20. Progression of Electrical conductivity (EC) in *Dolichos* stover compost over time



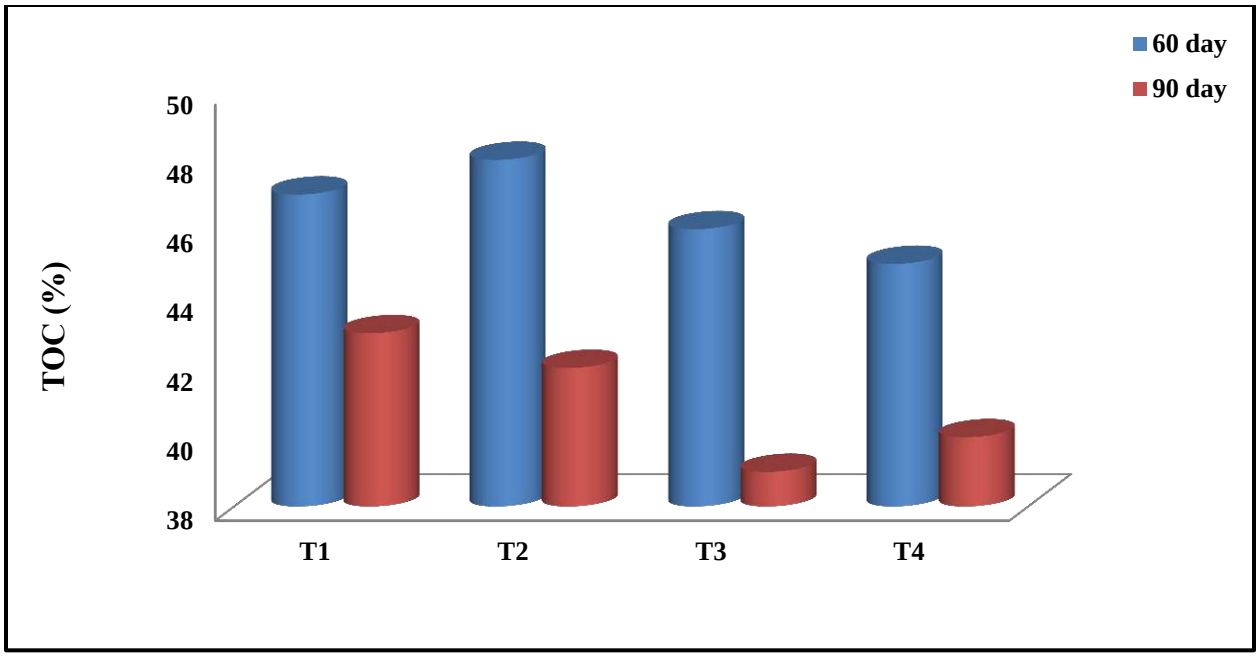
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.21. Progression of moisture content in *Dolichos* stover compost over time



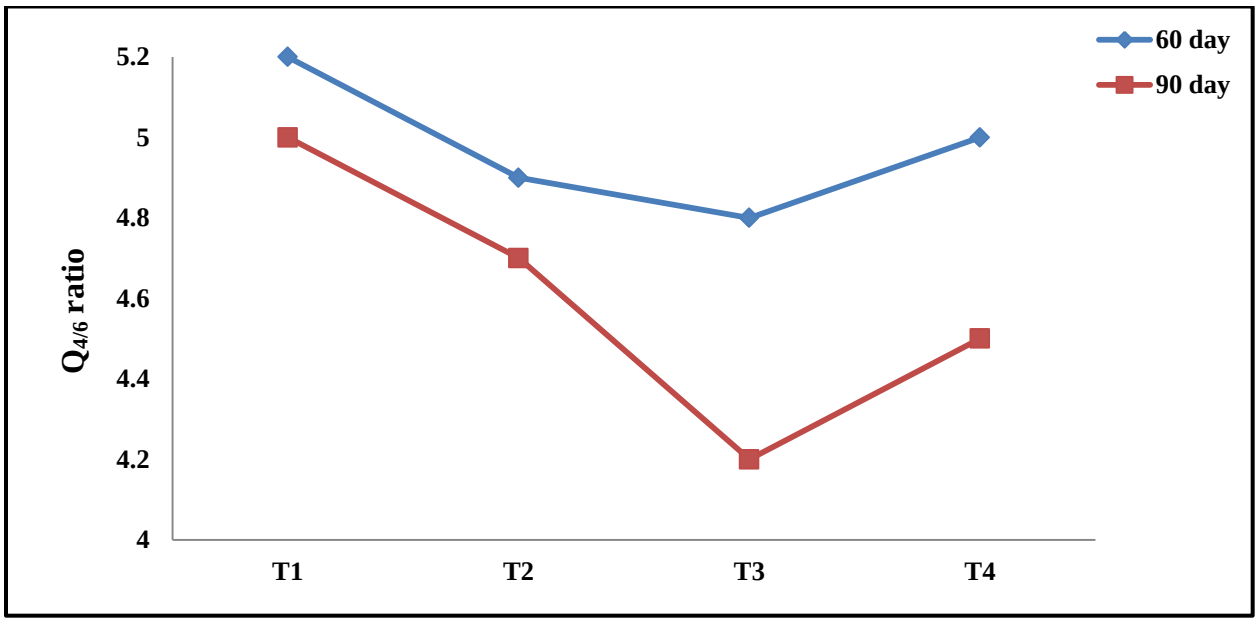
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.22. Progression of Total Organic Matter (TOM) in *Dolichos* stover compost over time



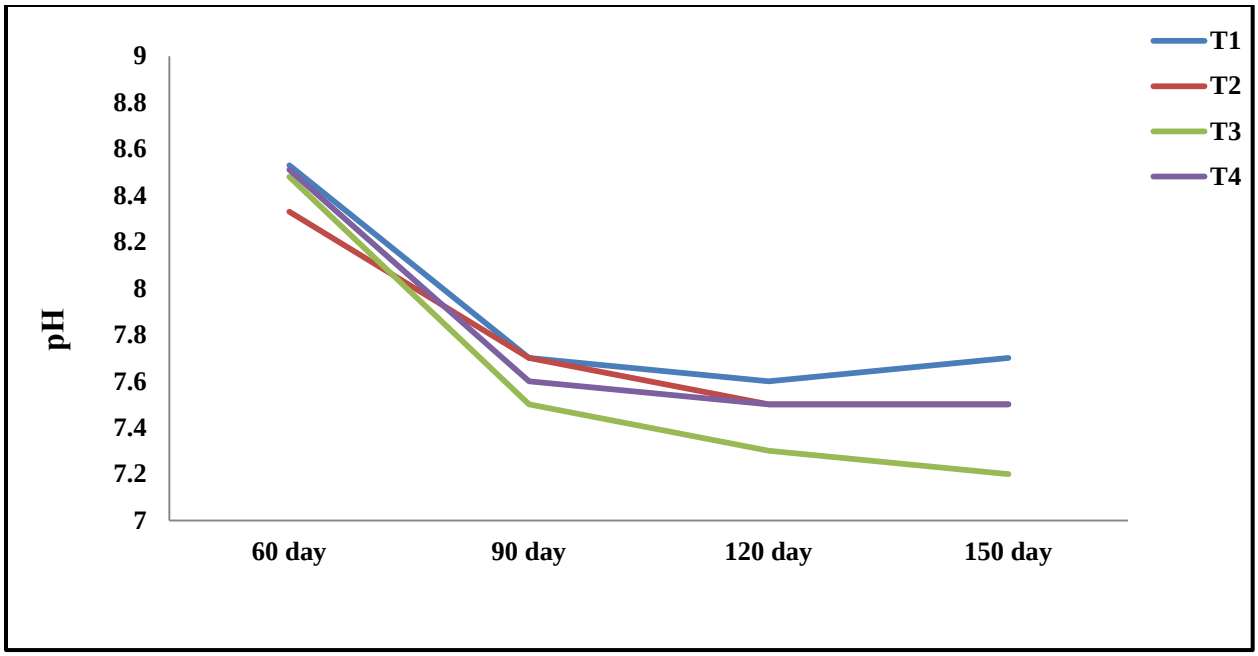
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.23. Progression of Total Organic Content (TOC) in *Dolichos* stover compost over time



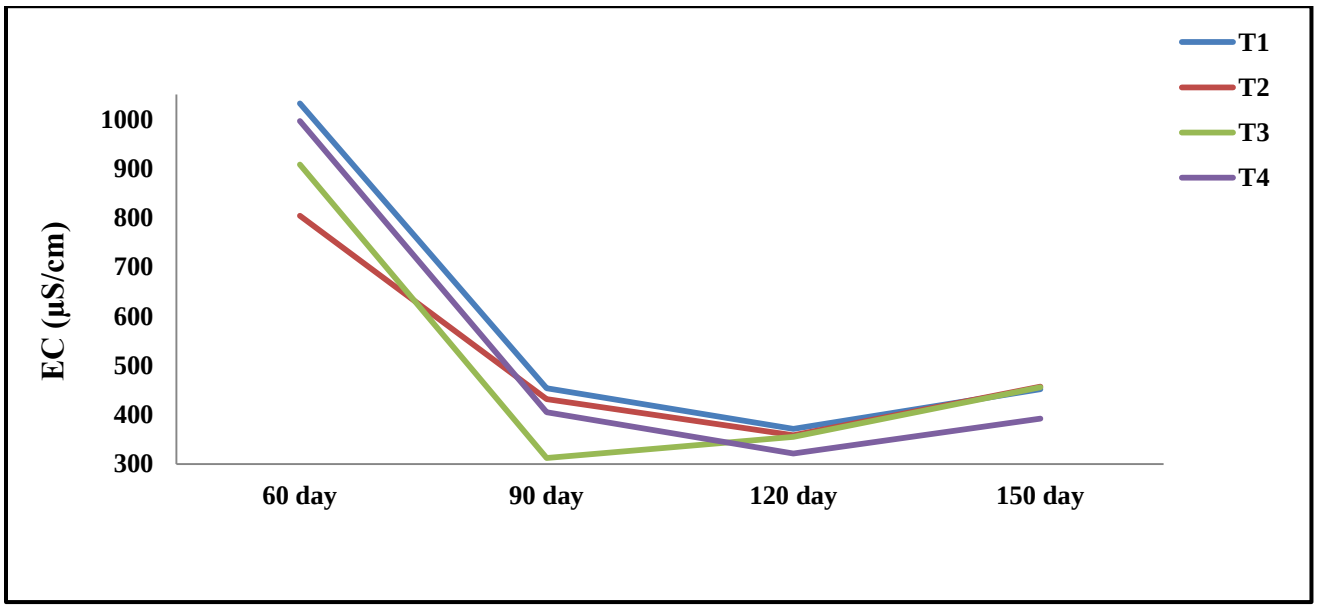
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.24. Progression of Humification Index (HI) in *Dolichos* stover compost over time



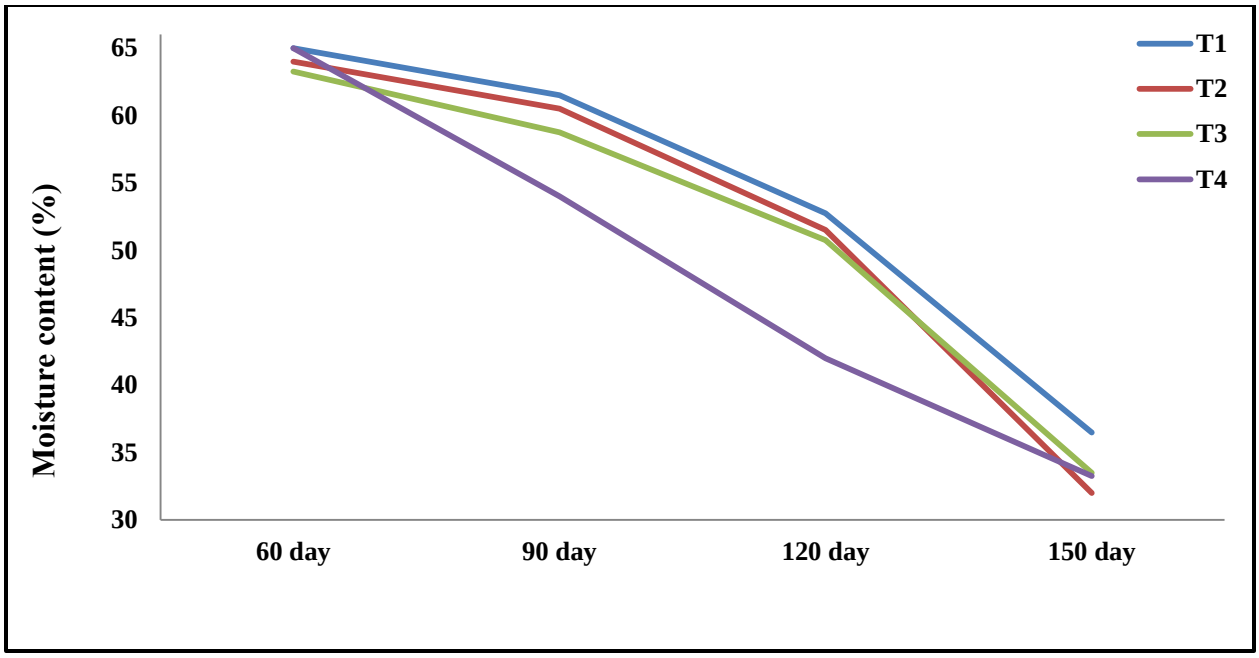
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.25. Progression of pH in chilli stalks compost over time



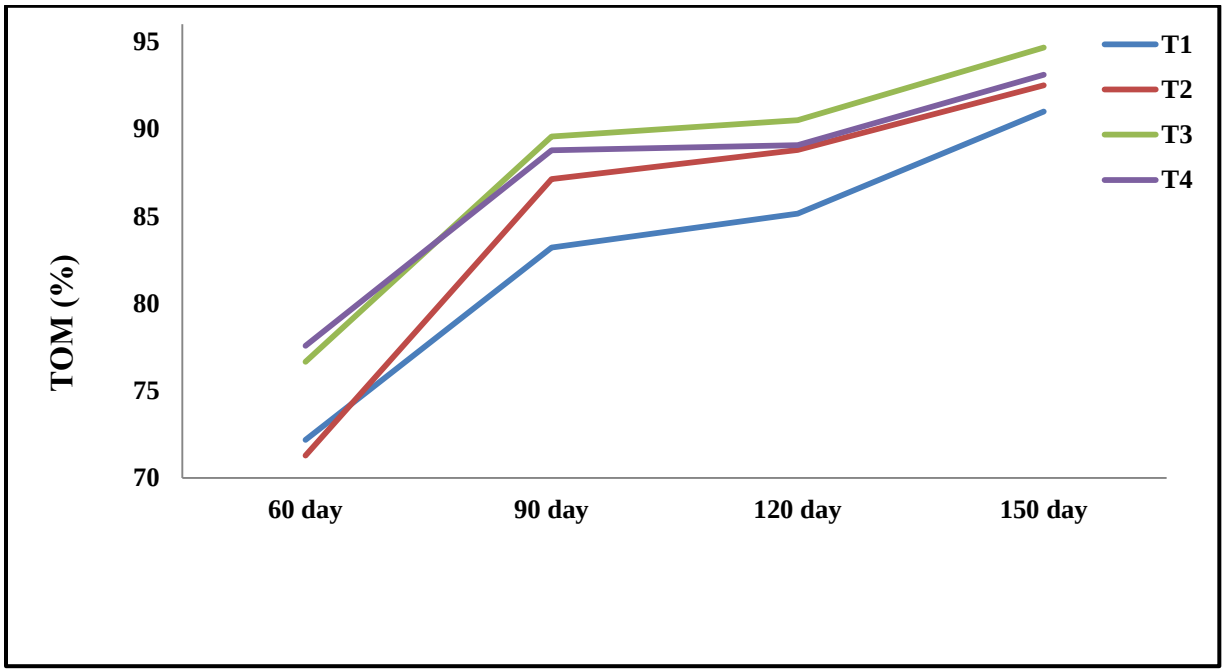
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.26. Progression of Electrical Conductivity (EC) in chilli stalks compost over time



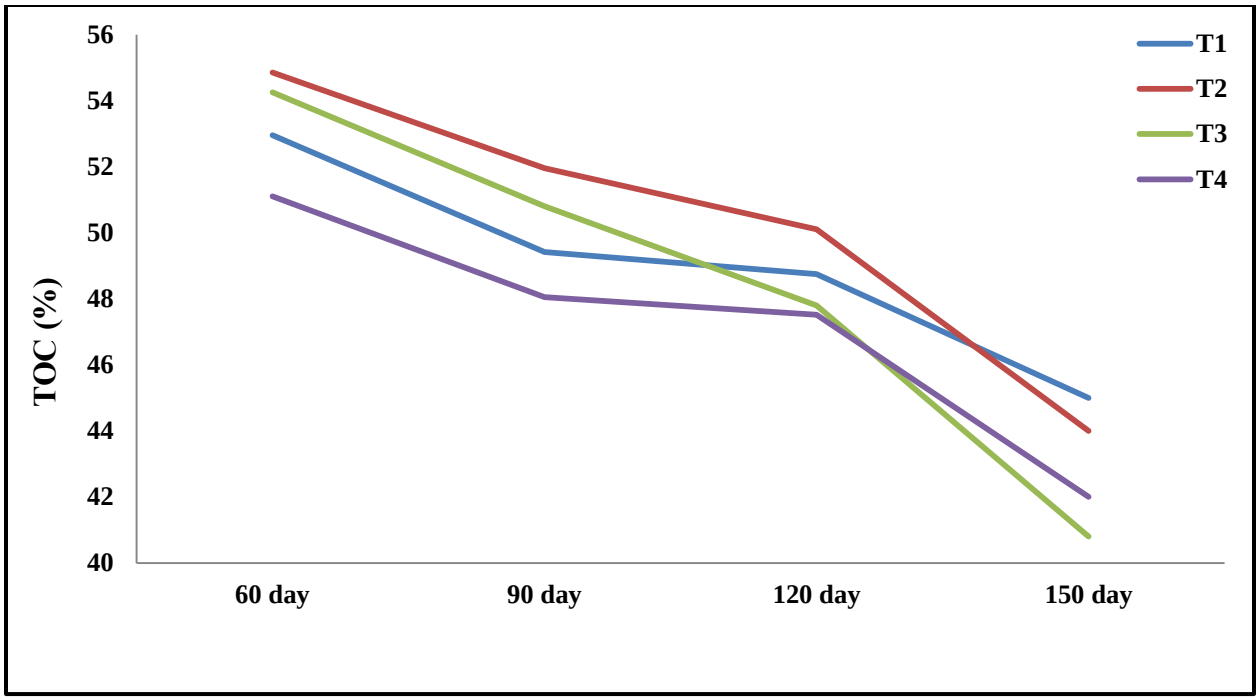
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.27. Progression of moisture content in chilli stalks compost over time



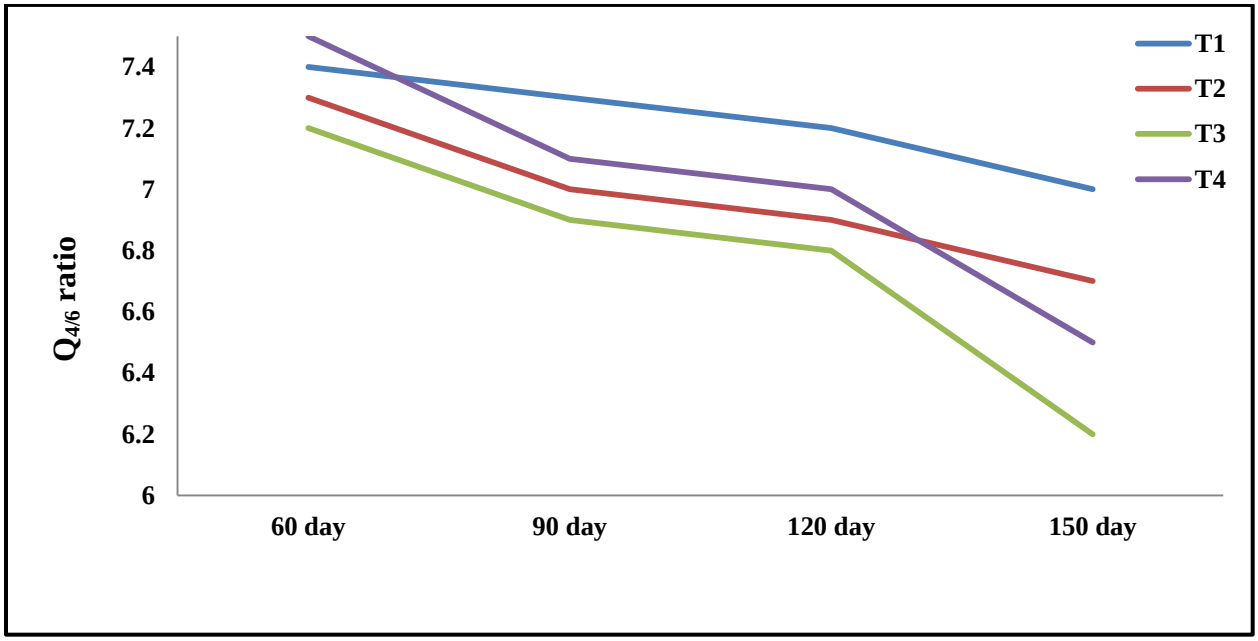
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.28. Progression of Total Organic Matter (TOM) in chilli stalks compost over time



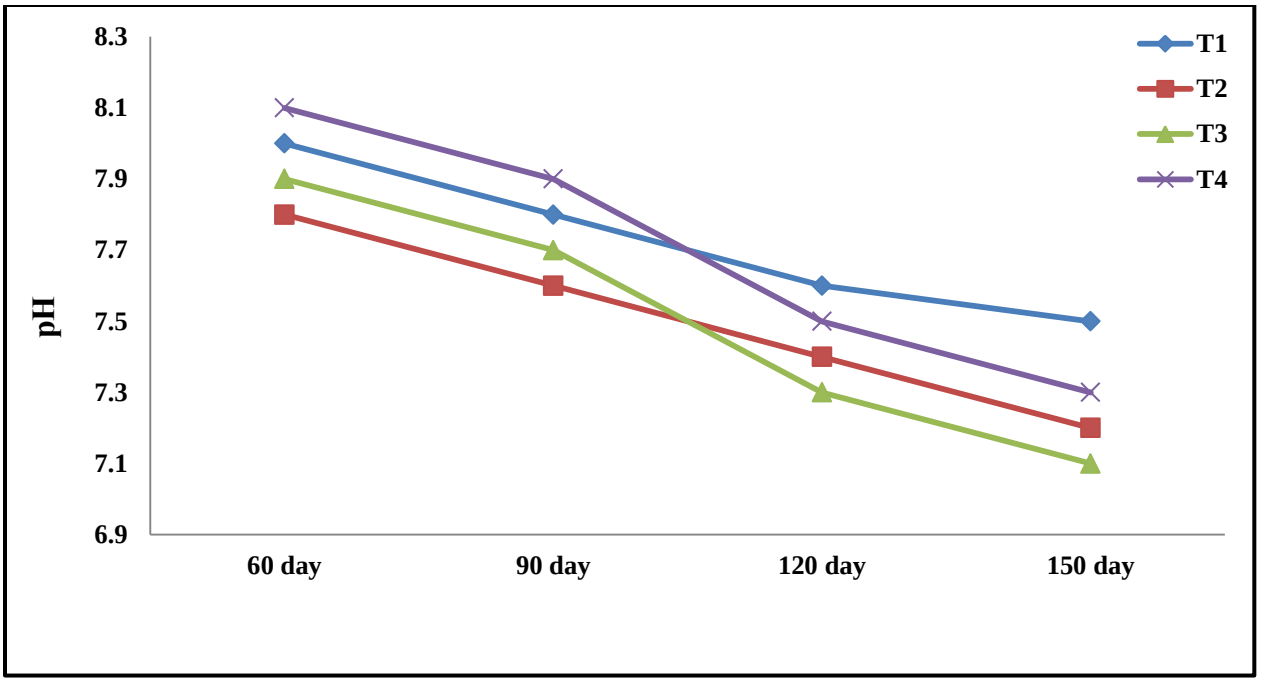
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.29. Progression of Total Organic Carbon (TOC) in chilli stalks compost over time



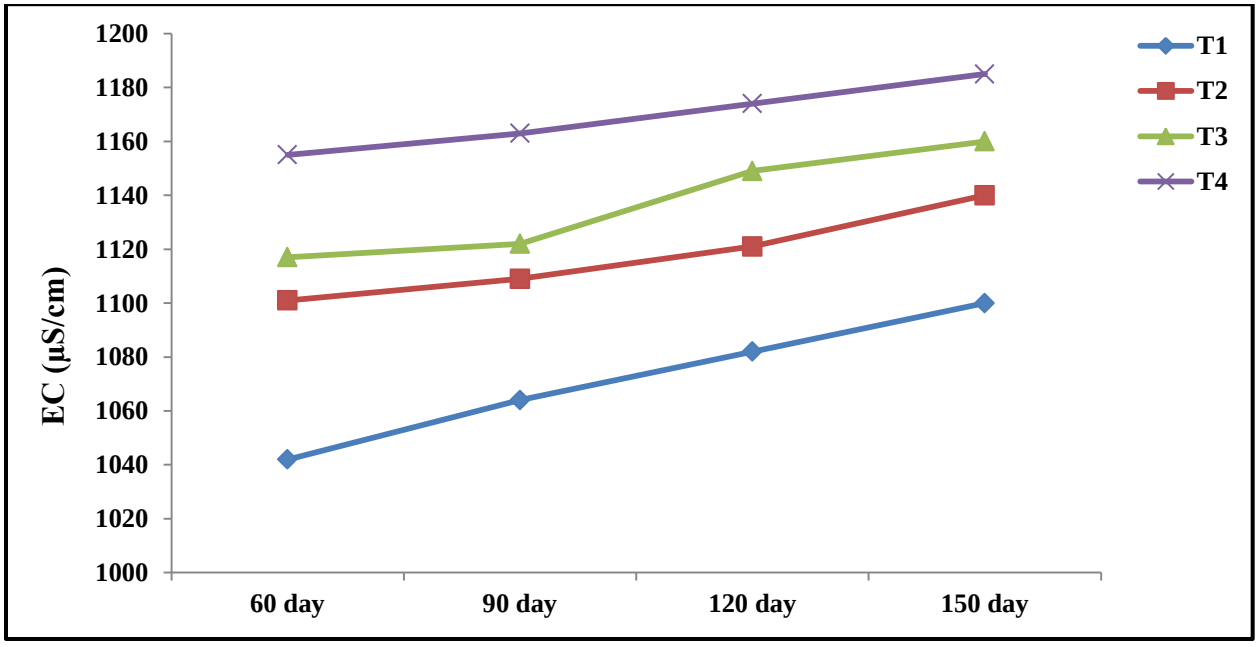
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.30. Progression of Humification Index (HI) in chilli stalks compost over time



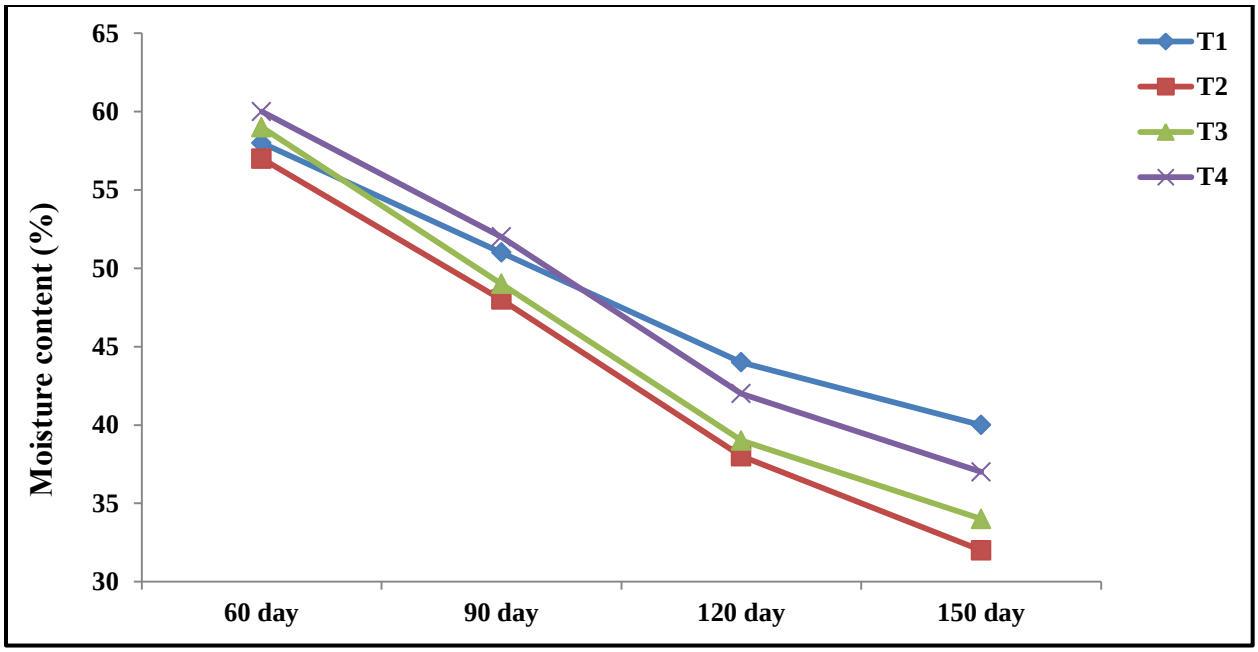
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.31. Progression of pH in grape pruning's compost over time



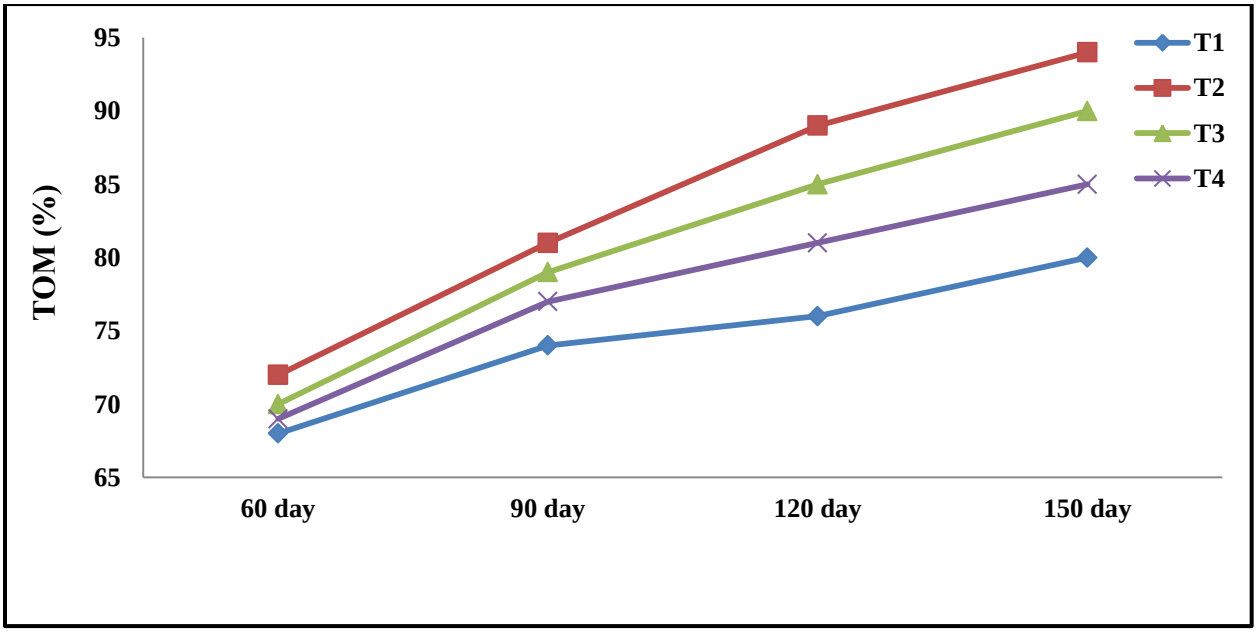
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.32. Progression of Electrical Conductivity (EC) in grape pruning's compost over time



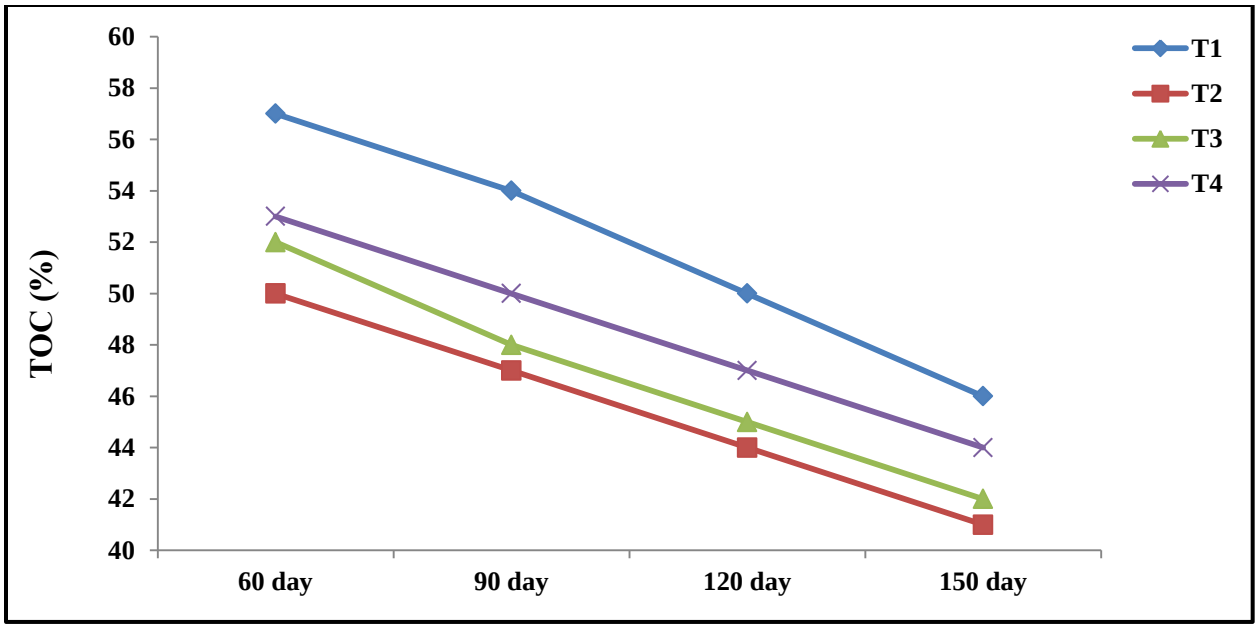
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.33. Progression of moisture content in grape pruning's compost over time



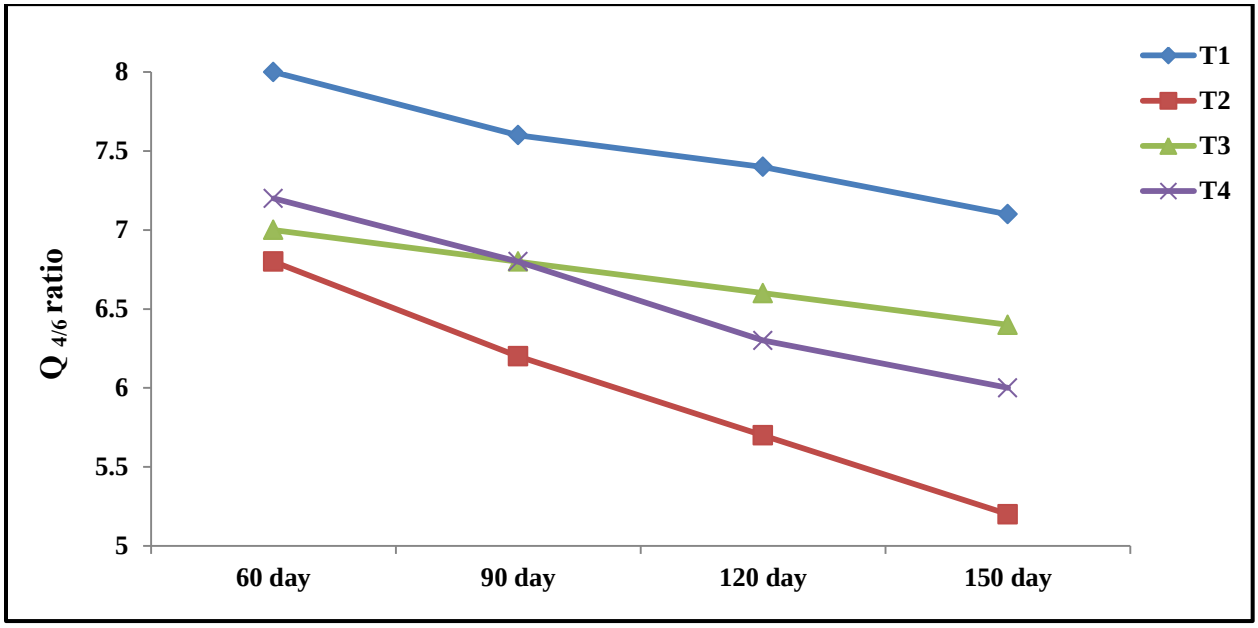
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.34. Progression of Total Organic Matter (TOM) in grape pruning's compost over time



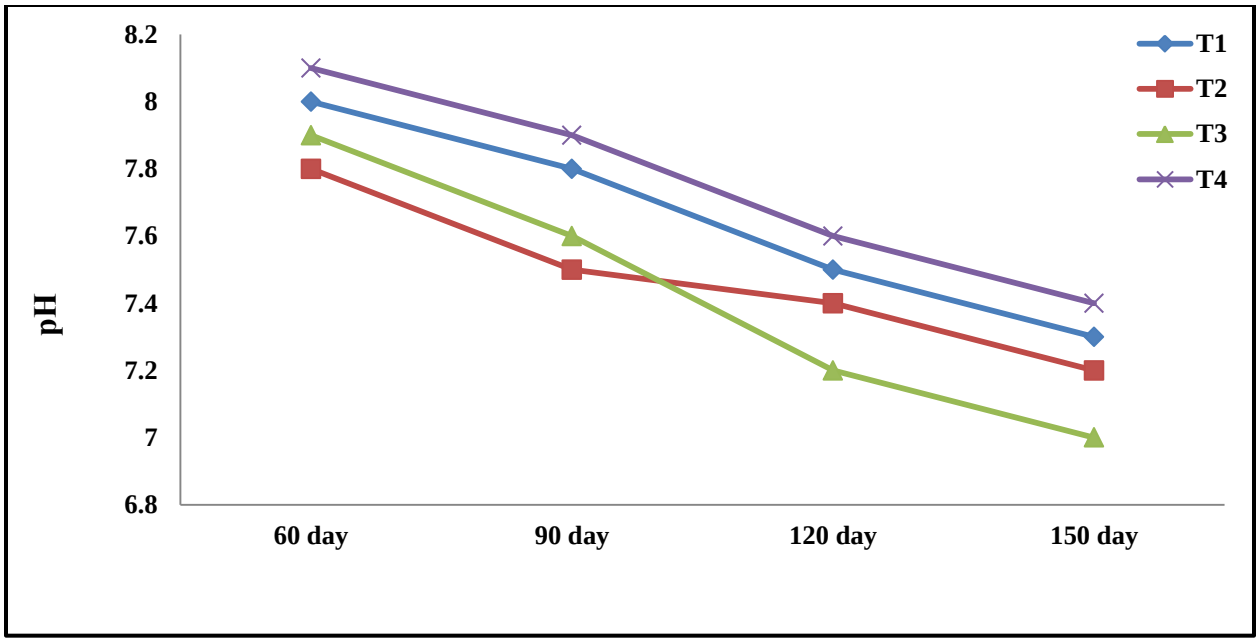
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.35. Progression of Total Organic Carbon (TOC) in grape Pruning's compost over time



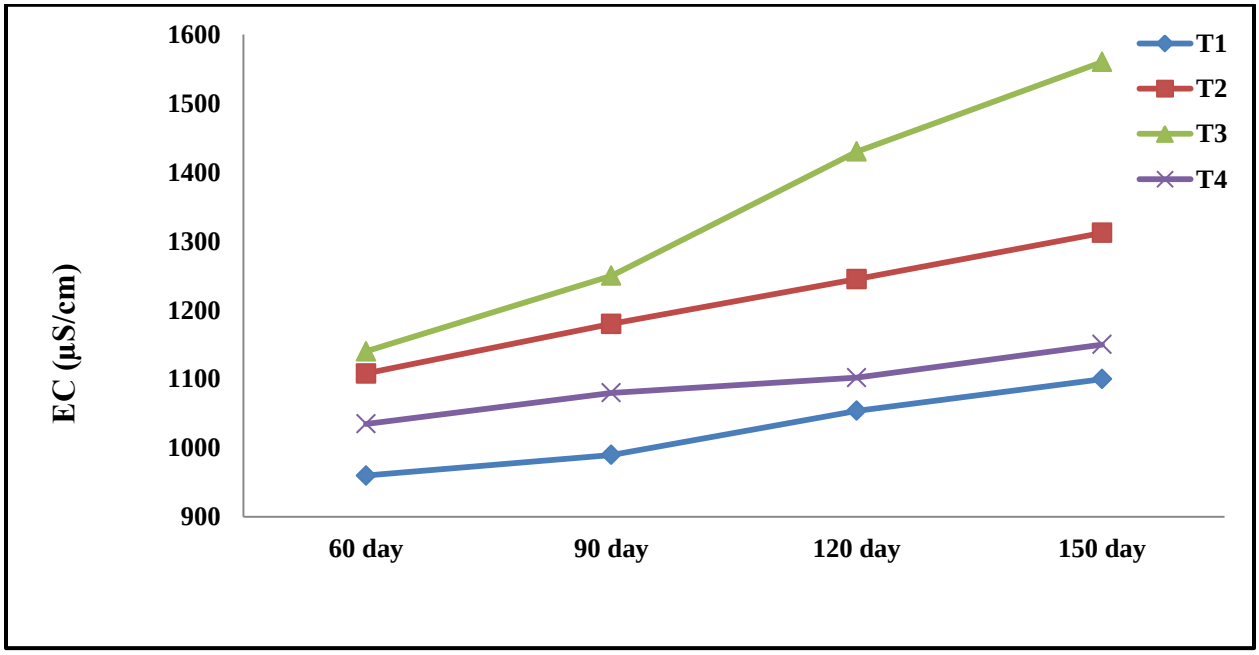
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.36. Progression of Humification Index (HI) in grape pruning's compost over time



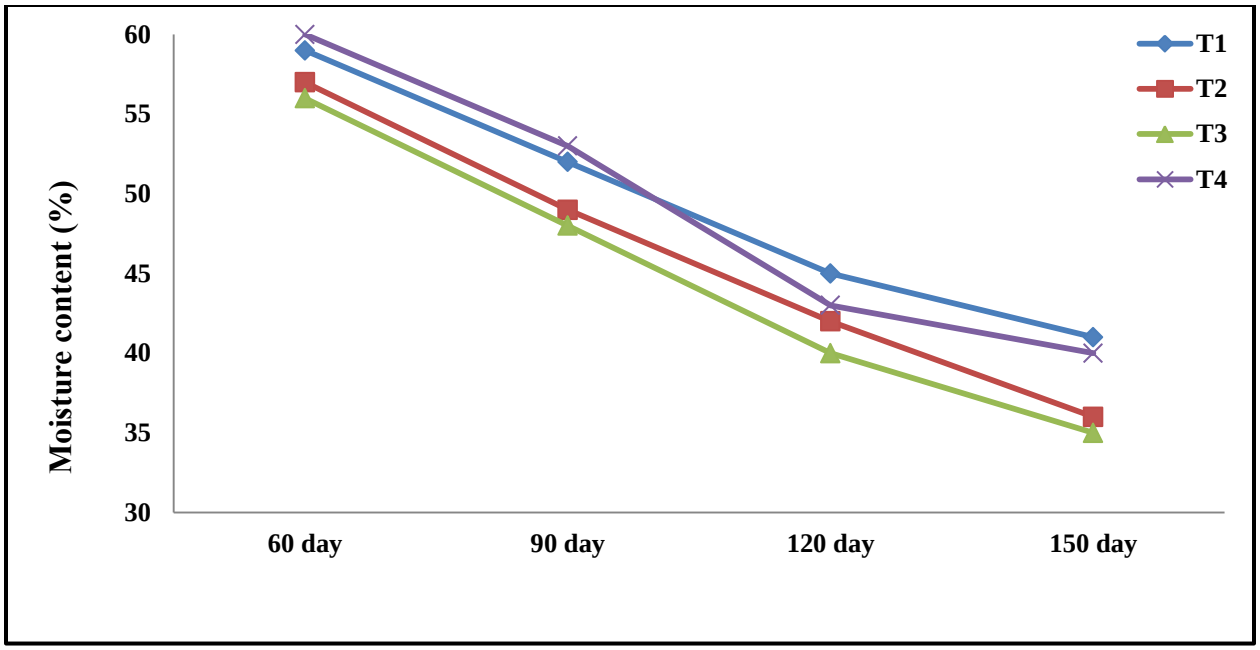
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.37. Progression of pH in brinjal stalks compost over time



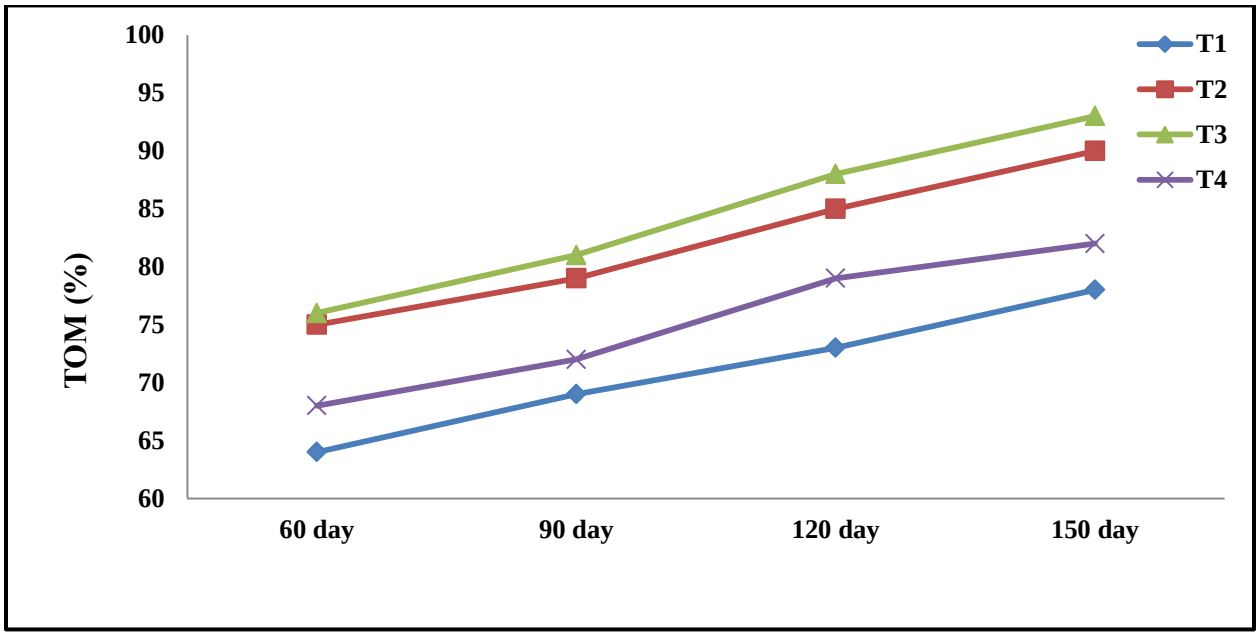
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.38. Progression of Electrical Conductivity (EC) in brinjal stalks compost over time



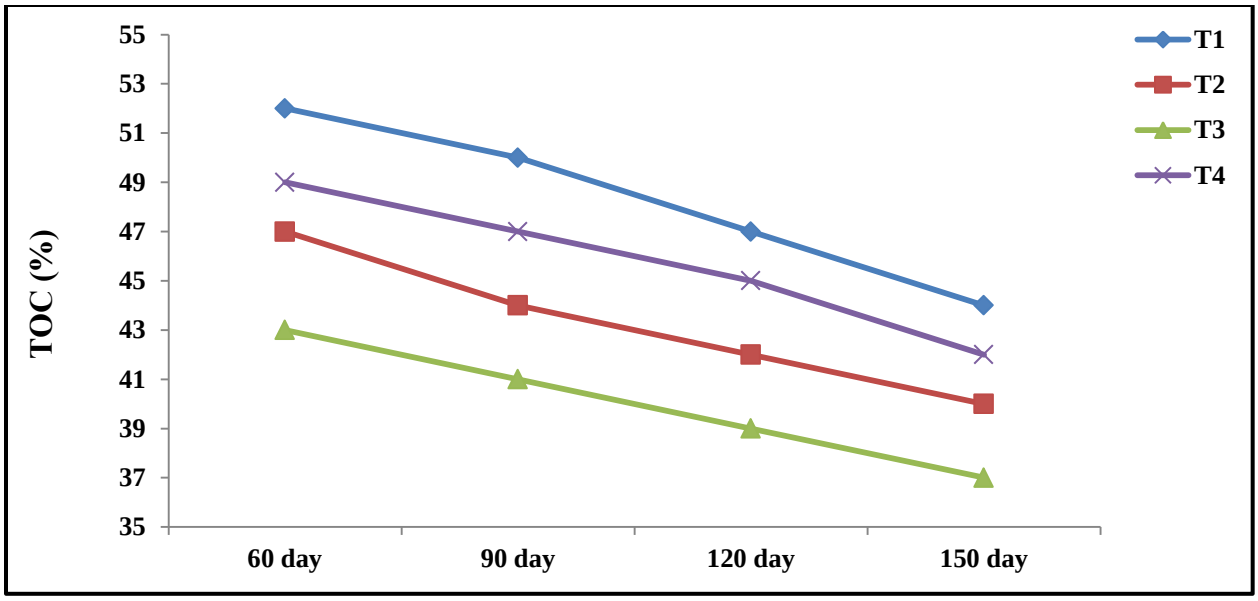
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.39. Progression of moisture content in brinjal stalks compost over time



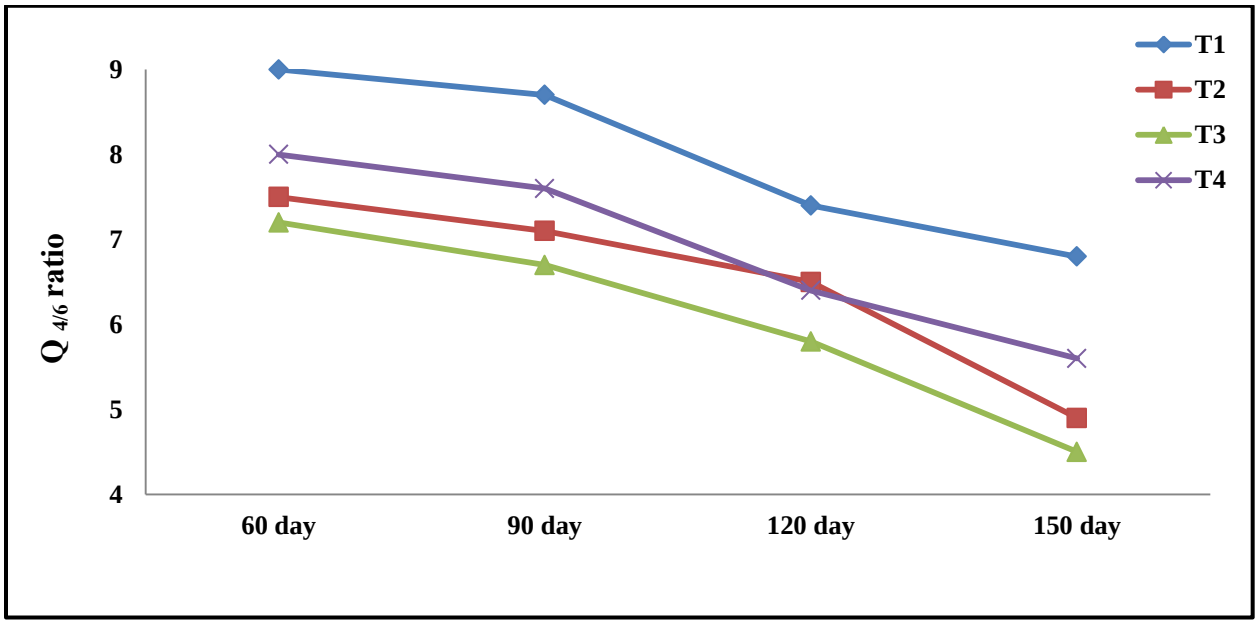
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.40. Progression of Total Organic Matter (TOM) in brinjal stalks compost over time



T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.41. Progression of Total Organic Carbon (TOC) in brinjal stalks compost over time



T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.42. Progression of Humification Index (HI) of brinjal stalks compost over time

4.8. Evaluation of compost stability and maturity of the prepared composts

Mango leaf litter compost prepared using the Consortium 1, possessed a humification index of 4.1, C:N ratio of 15.52, Humic like Carbon content of 171 g/kg, Fulvic like Carbon content of 51 g/kg, Humic like Carbon/ Fulvic like Carbon(HC/FC) ratio of 3.3, TOM content of 88%, TOC-40 %, WSC of 3.5 g/kg, WSN of 0.56 g/kg, WSC/WSN 6.3, C_{ext} of 62 g/kg, WSC/TOC of 0.109 (Table 4.13). When tested for its maturity, radish seeds had germination 90% and a germination index of 371.58, which were indicative of good stability and maturity (Figure 4.43).

Dolichos stover compost prepared using the Consortium 2, possessed a humification index of 4.2, C: N ratio of 14.82, Humic like Carbon content of 144 g/kg, Fulvic like Carbon content of 46 g/kg, Humic like Carbon/ Fulvic like Carbon (HC/FC) ratio of 3.13, TOM content of 92%, TOC-39%, WSC of 2.4 g/kg, WSN of 0.49 g/kg, WSC/WSN 4.89, C_{ext} of 103 g/kg, WSC/TOC of 0.05 (Table 4.14). When tested for its maturity radish seeds had germination 90% and a germination index of 224, which were indicative of good stability and maturity (Figure 4.44).

Chilli stalks compost prepared using the Consortium 2, possessed a humification index of 6.25, C:N ratio of 16.13, Humic like Carbon content of 20.5 g/kg, Fulvic like Carbon content of 69 g/kg, Humic like Carbon/ Fulvic like Carbon(HC/FC) ratio of 6.09, TOM content of 94%, TOC-41%, WSC of 3.2 g/kg, WSN of 0.56 g/kg, WSC/WSN 5.67, C_{ext} of 60.25 g/kg, WSC/TOC of 0.008 (Table 4.15). When tested for its maturity radish seeds had germination 90% with a germination index of 308.2, which were indicative of good stability and maturity (Figure 4.45).

Grape pruning's compost prepared using the Consortium 1, possessed a humification index of 5.2, C:N ratio of 14.4, Humic like Carbon content of 174 g/kg, Fulvic like Carbon content of 51 g/kg, Humic like Carbon/ Fulvic like Carbon(HC/FC) ratio of 3.41, TOM content of 94%, TOC-41%, WSC of 3.2 g/kg, WSN of 0.52 g/kg, WSC/WSN 6, C_{ext} of 33 g/kg, WSC/TOC of 0.078 (Table 4.16). When tested for its maturity radish seeds had a germination 90% and a germination index of 431.51, which were indicative of good stability and maturity (Figure 4.46).

Brinjal stalks compost prepared using the Consortium 2, possessed a humification index of 4.5, C: N ratio of 14.40, Humic like Carbon content of 204 g/kg, Fulvic like Carbon content of 44 g/kg, Humic like Carbon/ Fulvic like Carbon (HC/FC) ratio of 4.63, TOM content of 93%, TOC-37%, WSC of 4.6 g/kg, WSN of 0.33 g/kg, WSC/WSN 13.69, C_{ext} of 165 g/kg, WSC/TOC of 0.124 (Table 4.17). When tested for its maturity radish seeds had germination of 90% and a germination index of 308.21, which were indicative of good stability and maturity (Figure 4.47).

The Consortium-1 comprising (*Aspergillus sp.* TMLF-1, *Aspergillus oryzae* CP-2, *Trichoderma hamatum* FLF-13 and *Aspergillus sp.* TL-8), was found suitable for the bioconversion of mango leaf litter and grape pruning's. While the Consortium-2 comprising (*Bacillus endophyticus* FLCB-11, *Aspergillus sp*. CF-11, *Xylaria* sp. TF-4, *Penicillium chrysogenum* VCLF-1) was suitable for the bioconversion of *Dolichos* stover, chilli stalks and brinjal stalks.

Table 4.13. Stability and maturity parameters of mango leaf litter compost (90 DAI)

Trt	TOM (%)	TOC (%)	WSC (g/kg)	WSN (g/kg)	$Q_{4/6}$	C_{ext} (g/kg)	WSC/ WSN	HC (g/kg)	FC (g/kg)	HC/FC	WSC/TOC	pH	EC (µS/cm)	C:N
T_1	74	49	7.0	0.30	6.5	36	23.3	39	57	0.68	0.17	7.2	832	23.00
T_2	88	40	3.5	0.56	4.1	62	6.3	171	51	3.3	0.10	6.9	1200	15.52
T_3	82	46	4.0	0.46	4.9	54	8.7	53	60	0.88	0.11	7	1050	19.67
T_4	79	39	4.5	0.44	5.8	42	10.2	76	64	1.18	0.12	7.4	940	21.18
CV(%)	2.2	5.1	3.8	4.1	3.4	3.7	3.4	2.1	3.1	2.7	1.5	2.6	0.1	9.1
SE(d)	1.29	1.29	0.12	0.01	0.12	1.29	0.29	1.29	1.29	0.03	0.001	0.13	1.29	1.29
LSD at 1%	2.73	2.72	0.28	0.027	0.27	2.74	0.16	2.75	2.72	0.06	0.002	0.29	2.74	2.74

T_1- Uninoculated control; T_2-Consortium-1; T_3-Consortium-2; T_4-Consortium-3

DAI-Days after inoculation; TOM-Total Organic Matter; TOC-Total Organic Carbon; WSC-Water Soluble Carbon; WSN-Water Soluble Nitrogen; Q4/6-Humification Index; C_{ext}-Alkali extractable carbon; HC-Humic like carbon; FC-Fulvic like carbon; EC-Electrical conductivity; C:N-Carbon to Nitrogen ratio.

Table 4.14. Stability and maturity parameters of *Dolichos* stover compost (90 DAI)

Trt	TOM (%)	TOC (%)	WSC (g/kg)	WSN (g/kg)	$Q_{4/6}$	C_{ext} (g/kg)	WSC/WSN	HC (g/kg)	FC (g/kg)	HC/FC	WSC/TOC	pH	EC (µS/cm)	C:N
T_1	89	43	6.0	0.2	5.0	87	20.6	108	78	1.3	0.12	7.5	1055	20
T_2	91	42	3.5	0.3	4.7	50	9.4	109	63	1.7	0.07	7.5	1100	15.2
T_3	92	39	2.4	0.4	4.2	103	4.8	144	46	3.1	0.05	7.2	1120	14.8
T^4	90	40	2.7	0.4	4.5	75	6.0	72	31	2.3	0.05	7.6	1110	15.3
CV(%)	2.0	4.4	25.3	4.5	3.7	2.3	24.6	1.6	3.3	2.7	22.3	2.4	1.3	5.5
SE(d)	1.29	1.29	0.66	0.01	0.12	1.2	1.77	1.29	1.29	0.04	0.01	0.12	10.66	0.64
LSD at 1%	NS	2.75	1.39	0.02	0.25	2.74	3.77	2.74	2.72	0.08	0.02	0.28	22.57	1.36

T_1- Uninoculated control; T_2-Consortium-1; T_3-Consortium-2; T_4-Consortium-3

DAI-Days after inoculation; TOM-Total Organic Matter; TOC-Total Organic Carbon; WSC-Water Soluble Carbon; WSN-Water Soluble Nitrogen; Q4/6-Humification Index; C_{ext}-Alkali extractable carbon; HC-Humic like carbon; FC-Fulvic like carbon; EC-Electrical conductivity; C:N-Carbon to Nitrogen ratio.

Table 4.15. Stability and maturity parameters of chilli stalks compost (150 DAI)

Trt	TOM (%)	TOC (%)	WSC (g/kg)	WSN g/kg)	$Q_{4/6}$	C_{ext} (g/kg)	WSC/WSN	HC (g/kg)	FC (g/kg)	HC/FC	WSC/TOC	pH	EC (µS/cm)	C:N
T_1	91	45	5.2	0.35	7.0	63	14.7	69	21	3.3	0.12	7.67	452	19.4
T_2	92	44	4.8	0.21	6.7	74	22.8	105	20	5.3	0.11	7.52	457	16.8
T_3	94	41	3.2	0.56	6.2	60	5.6	96	15.7	6.1	0.008	7.2	456	16.1
T_4	93	42	3.6	0.22	6.5	44	16.1	97	19	5.1	0.09	7.5	392	14.7
CV(%)	0.5	1.0	5.4	8.5	2.6	2.7	11.8	9.7	1.7	68.1	5.2	1.1	2.6	11.8
SE(d)	0.36	0.32	0.16	0.02	0.12	1.16	1.24	1.31	1.11	0.09	0.004	0.06	8.08	1.22
LSD at 1%	0.77	0.69	0.34	0.04	0.25	2.46	2.64	2.78	2.35	NS	0.009	0.13	17.14	2.62

T_1- Uninoculated control; T_2-Consortium-1; T_3-Consortium-2; T_4-Consortium-3

DAI-Days after inoculation; TOM-Total Organic Matter; TOC-Total Organic Carbon; WSC-Water Soluble Carbon; WSN-Water Soluble Nitrogen; Q4/6-Humification Index; C_{ext}-Alkali extractable carbon; HC-Humic like carbon; FC-Fulvic like carbon; EC-Electrical conductivity; C:N-Carbon to Nitrogen ratio.

Table: 4.16. Stability and maturity parameters of grape pruning's compost (150 DAI)

Trt	TOM (%)	TOC (%)	WSC (g/kg)	WSN (g/kg)	$Q_{4/6}$	C_{ext} (g/kg)	WSC/WSN	HC (g/kg)	FC (g/kg)	HC/FC	WSC/TOC	pH	EC (µS/cm)	C:N
T_1	78	44	7.0	0.23	6.8	90	30.4	90	80	1.12	0.15	7.3	1100	23.40
T_2	90	40	5.1	0.34	4.9	142	15.0	128	62	2.06	0.12	7.2	1312	16.30
T_3	93	37	4.6	0.33	4.5	165	13.6	204	44	4.63	0.12	7.0	1560	14.40
T_4	82	42	3.6	0.18	5.6	138	19.2	122	74	1.64	0.008	7.4	1150	20.20
CV(%)	1.90	4.06	3.13	5.6	3.03	1.23	4.06	1.19	2.57	2.97	1.12	2.28	0.13	0.89
SE(d)	0.95	0.95	0.95	0.009	0.09	0.95	0.47	0.95	0.95	0.04	0.001	0.09	0.95	0.09
LSD at 1%	2.00	2.01	0.201	0.01	0.20	2.02	0.98	2.01	2.02	0.09	0.002	0.19	2.00	0.19

T_1- Uninoculated control; T_2-Consortium-1; T_3-Consortium-2; T_4-Consortium-3

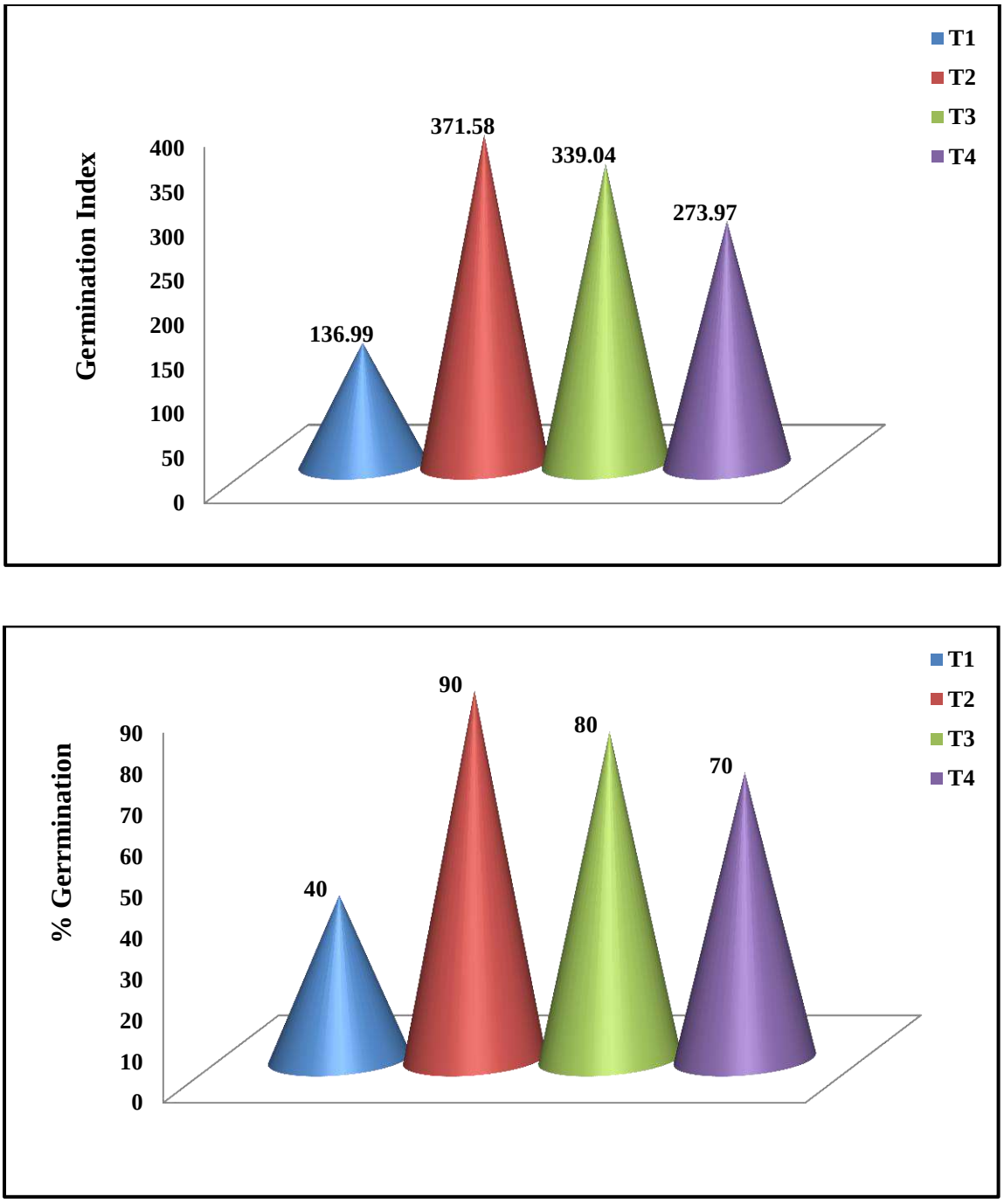
DAI-Days after inoculation; TOM-Total Organic Matter; TOC-Total Organic Carbon; WSC-Water Soluble Carbon; WSN-Water Soluble Nitrogen; Q4/6-Humification Index; C_{ext}-Alkali extractable carbon; HC-Humic like carbon; FC-Fulvic like carbon; EC-Electrical conductivity; C:N-Carbon to Nitrogen ratio.

Table: 4.17. Stability and maturity parameters of brinjal stalks compost (150 DAI)

Trt	TOM (%)	TOC (%)	WSC (g/kg)	WSN (g/kg)	$Q_{4/6}$	C_{ext} (g/kg)	WSC/WSN	HC (g/kg)	FC (g/kg)	HC/FC	WSC/TOC	pH	EC (µS/cm)	C:N
T_1	80	46	5.2	0.29	7.1	12	17.9	82	72	1.13	0.11	7.5	1100	24.0
T_2	94	41	3.2	0.52	5.2	33	6.0	174	51	3.41	0.07	7.2	1040	14.4
T_3	90	42	3.6	0.37	6.4	30	9.7	142	57	2.49	0.08	7.1	1160	15.6
T_4	85	44	4.8	0.31	6.0	27	15.4	106	64	1.65	0.10	7.3	1185	17.4
CV(%)	1.95	4.22	4.35	4.90	2.96	7.16	2.48	1.45	2.99	2.31	0.69	2.51	0.16	10.23
SE(d)	1.20	1.29	0.12	0.01	0.12	1.29	0.12	1.29	1.29	0.03	0.0001	0.12	1.29	1.29
LSD at 1%	2.56	2.72	0.27	0.02	0.27	2.74	0.46	2.72	2.73	0.07	0.0002	0.027	2.73	2.75

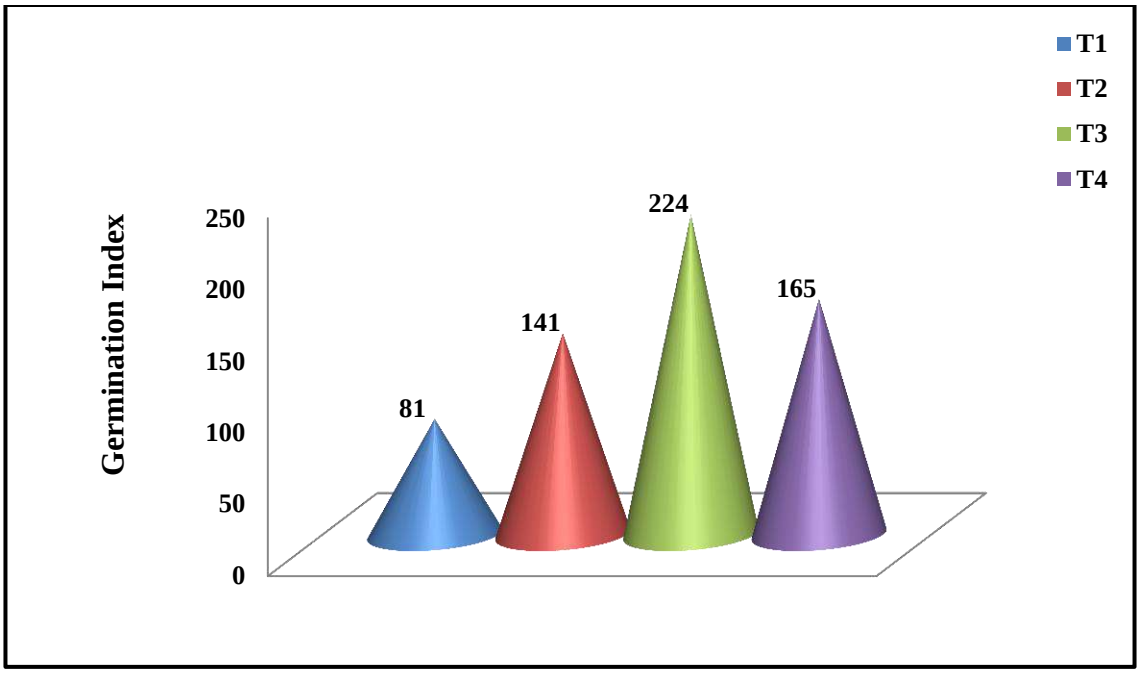
T_1- Uninoculated control; T_2-Consortium-1; T_3-Consortium-2; T_4-Consortium-3

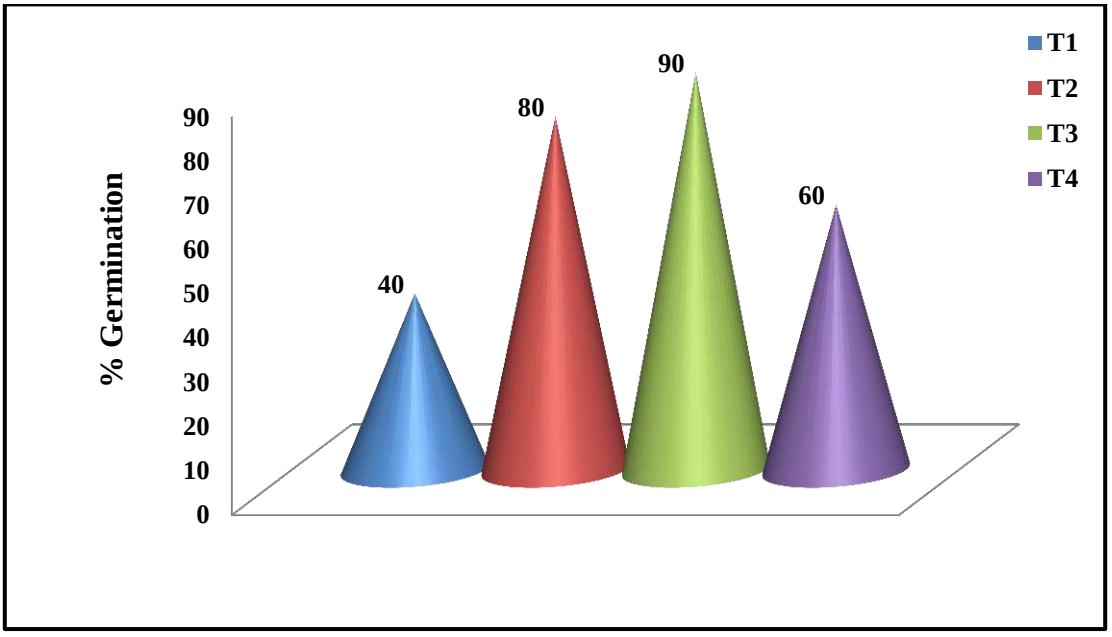
DAI-Days after inoculation; TOM-Total Organic Matter; TOC-Total Organic Carbon; WSC-Water Soluble Carbon; WSN-Water Soluble Nitrogen; $Q_{4/6}$-Humification Index; C_{ext}-Alkali extractable carbon; HC-Humic like carbon; FC-Fulvic like carbon; EC-Electrical conductivity; C:N-Carbon to Nitrogen ratio.



T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

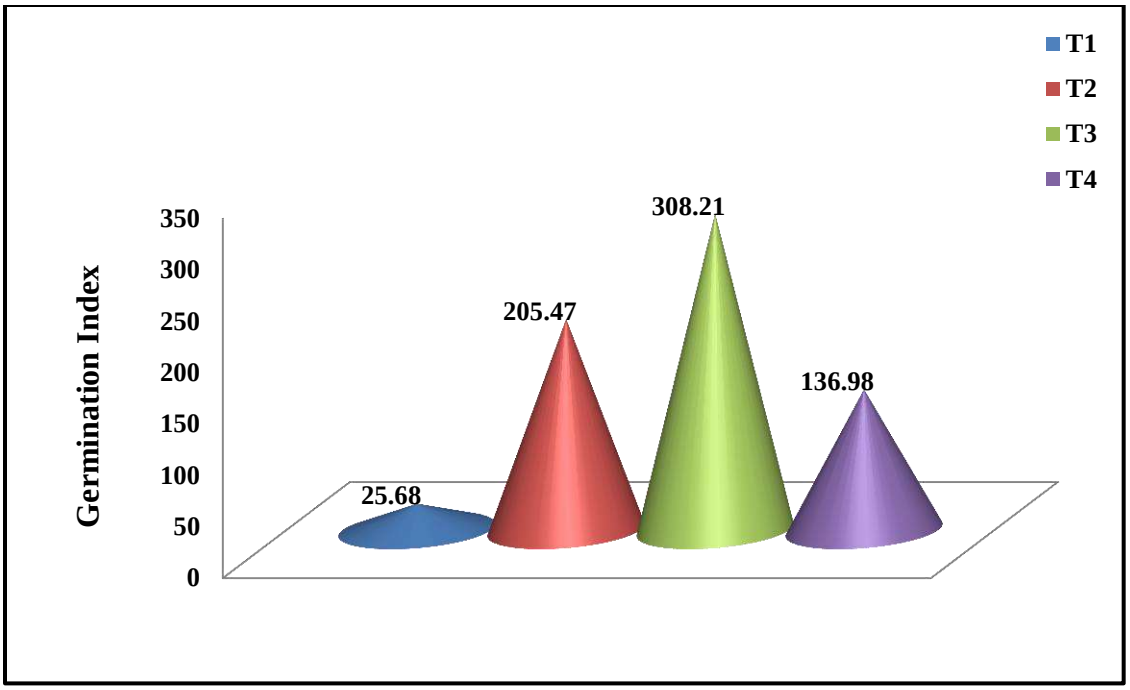
Figure 4.43. Compost maturity of mango leaf litter compost as determined by Germination Index (GI) and percent germination of radish seeds

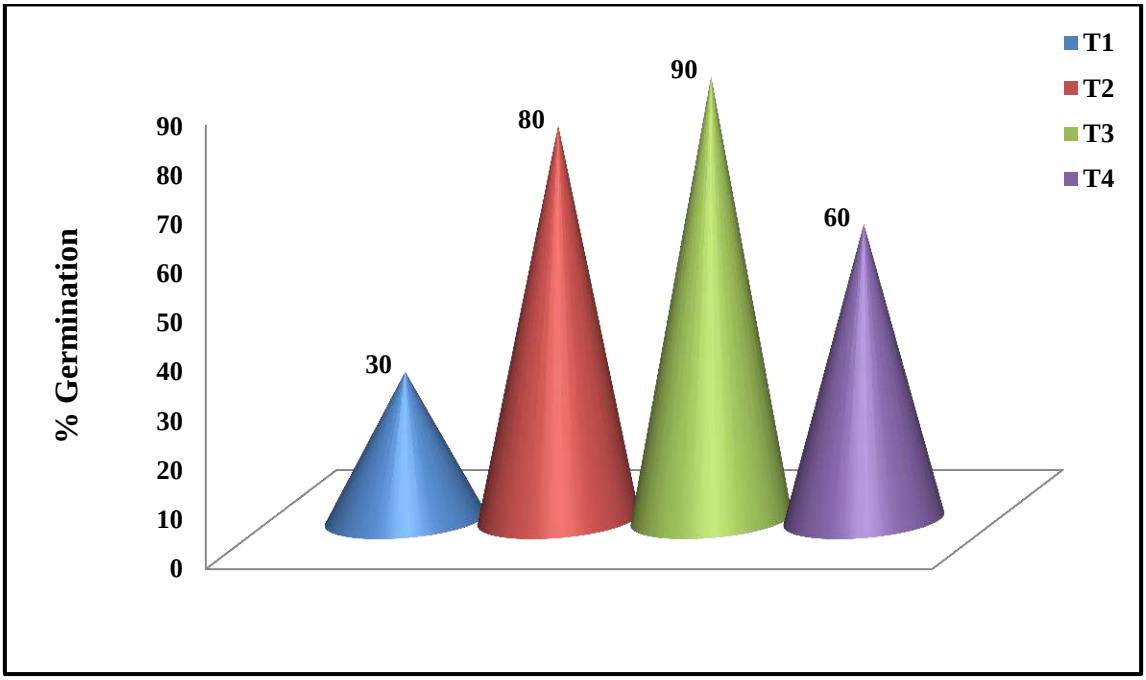




T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

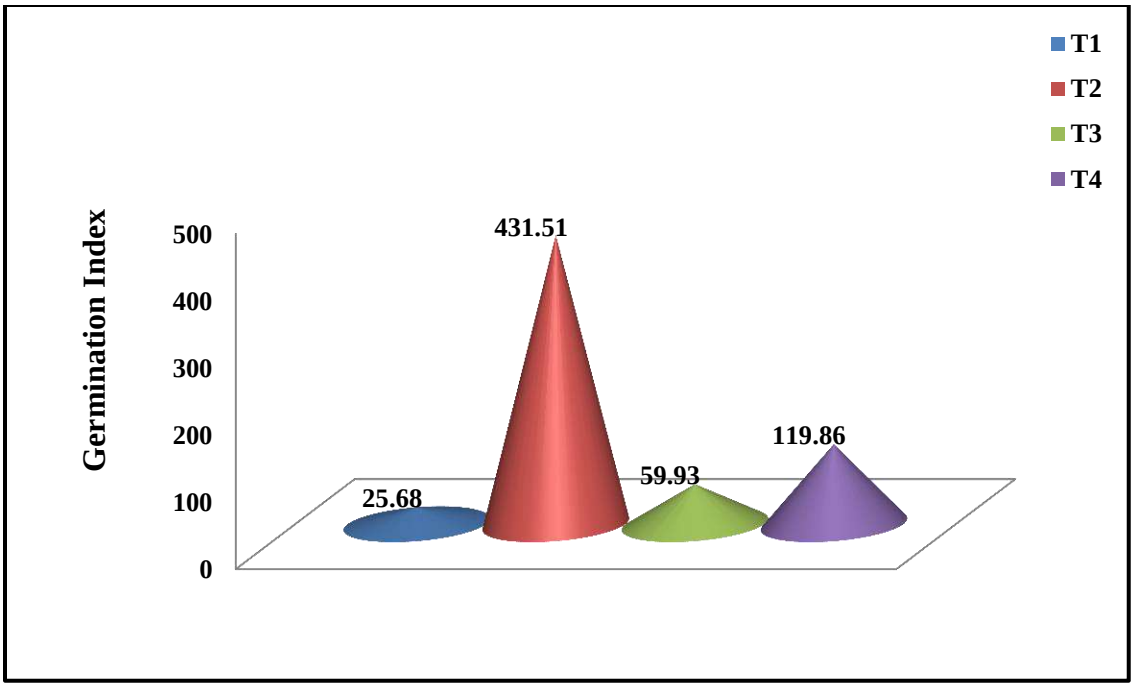
Figure 4.44. Compost maturity of *Dolichos* stover compost as determined by Germination Index (GI) and percent germination of radish seeds

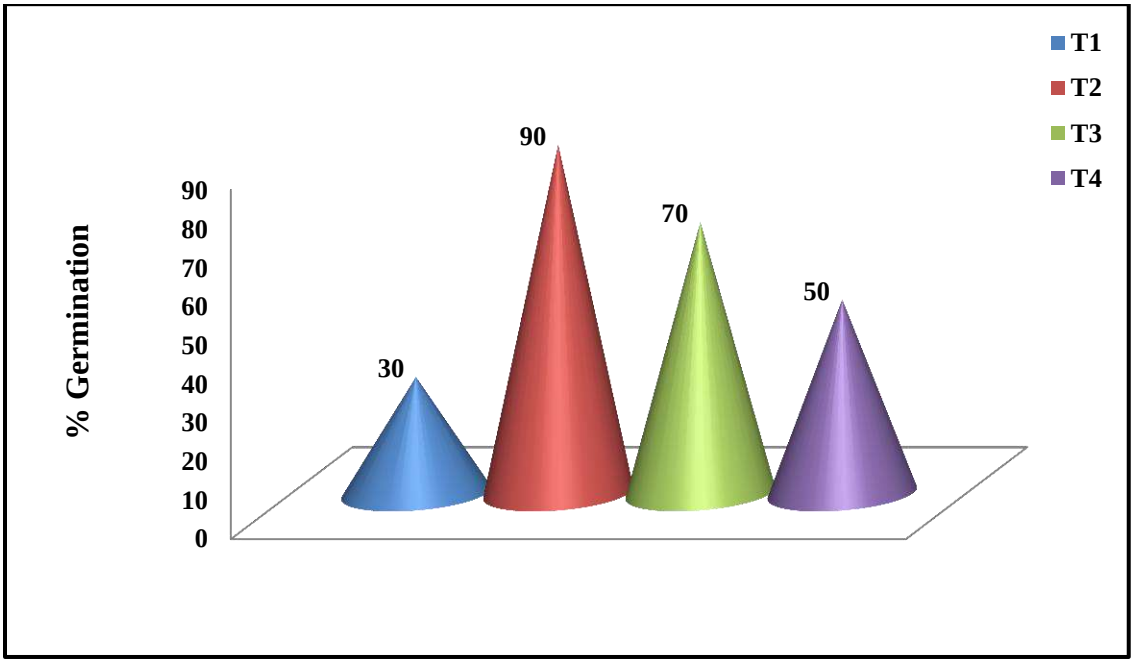




T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

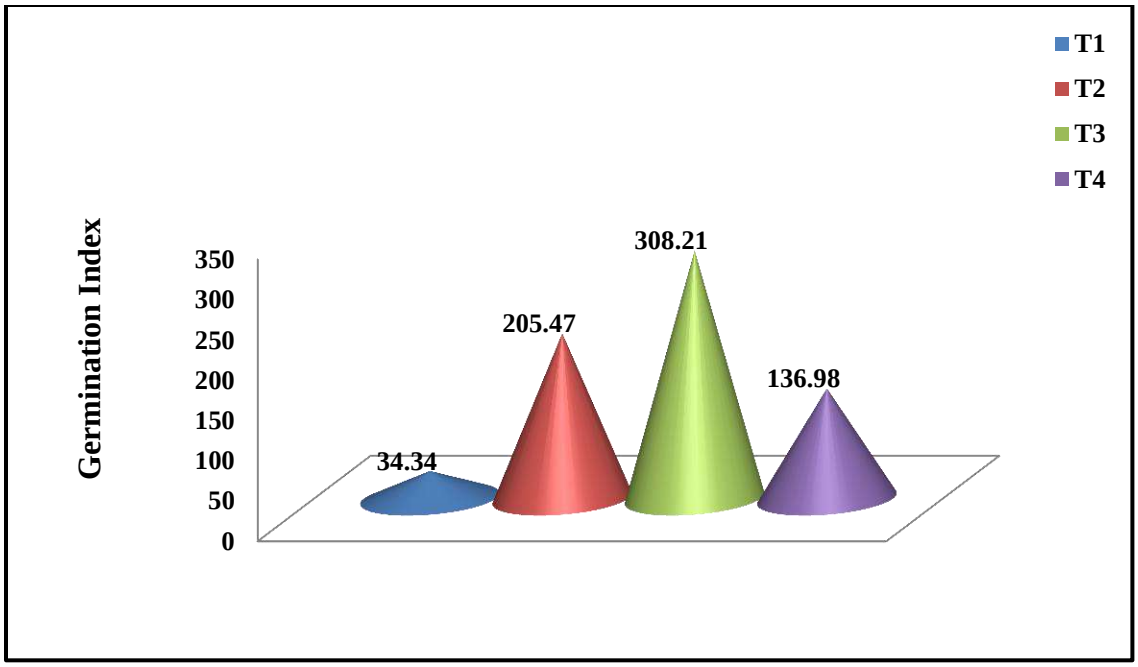
Figure 4.45. Compost maturity of chilli stalks compost as determined by Germination Index (GI) and percent germination of radish seeds

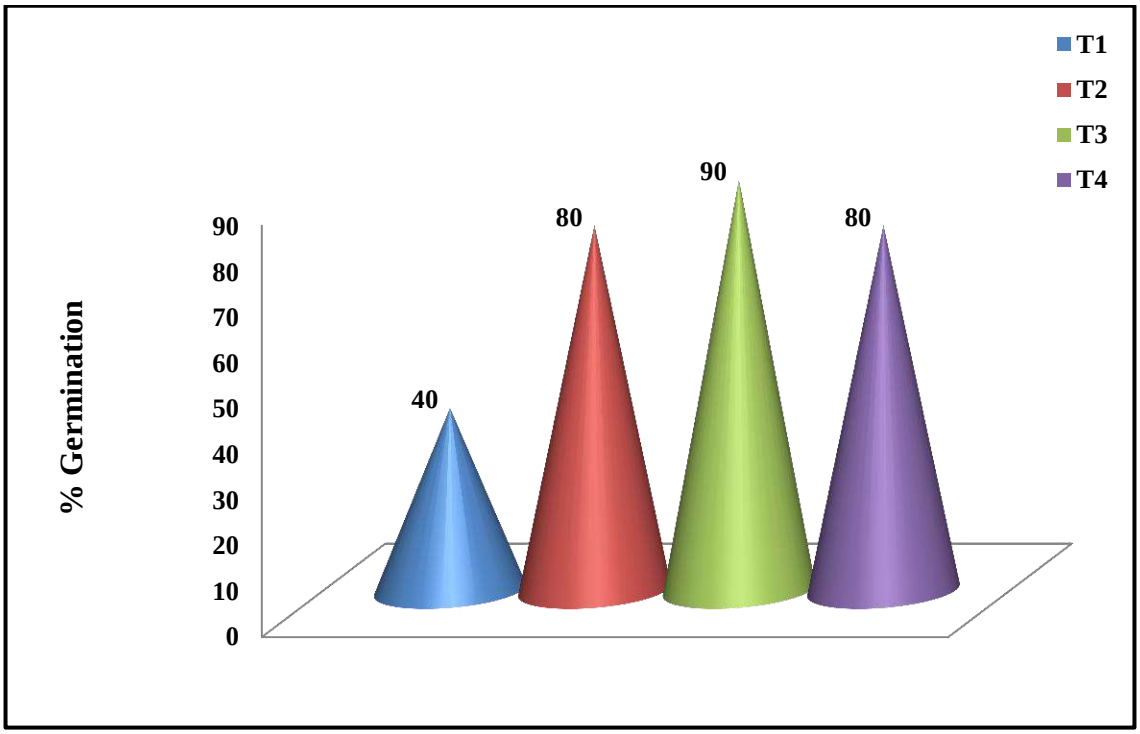




T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.46. Compost maturity of grape pruning's compost as determined by Germination Index (GI) and percent germination of radish seeds.





T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.47. Compost maturity of brinjal stalks compost as determined by Germination Index (GI) and percent germination of radish seeds

(A) Mango leaf litter

(A1) Mango leaf litter compost

(B) *Dolichos* stover

(B1) *Dolichos* stover compost

Plate 4.11. Substrates and their composts

(A) Chilli stalks

(A1) Chilli stalks compost

(B) Grape pruning's

(B1) Grape pruning's compost

Plate 4.12. Substrates and their composts

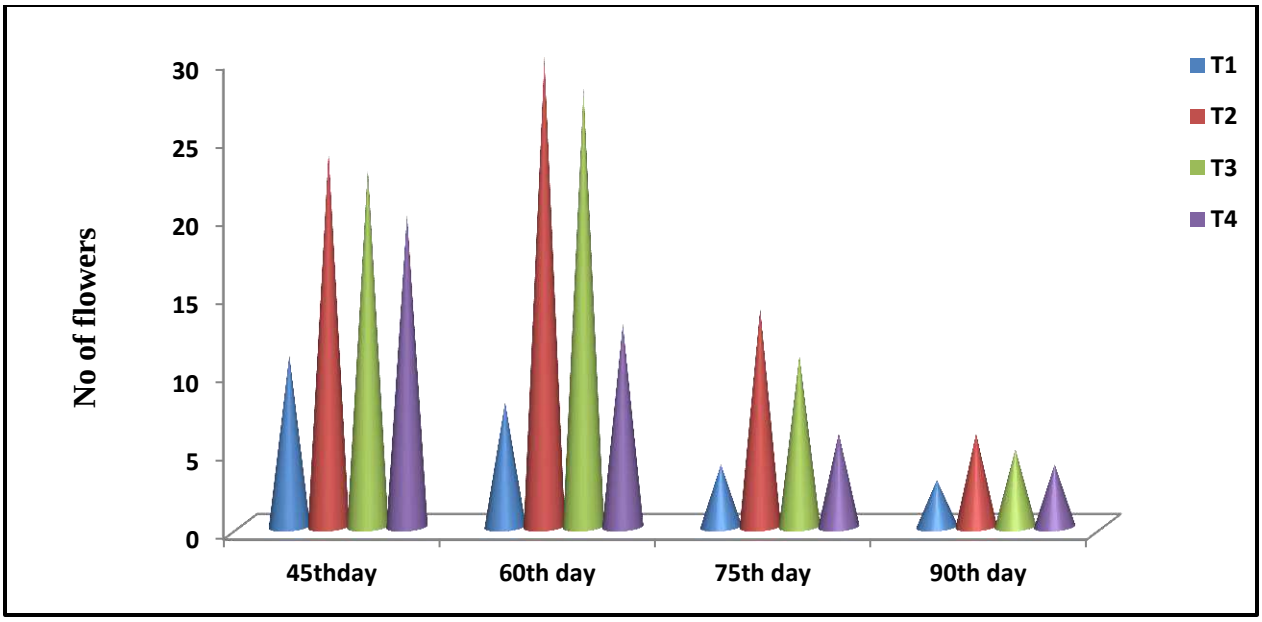
(A) Brinjal stalks **(A1) Brinjal stalks compost**

Plate 4.13. Substrates and their composts

4.9. Evaluation of the composts prepared on tomato under polyhouse conditions

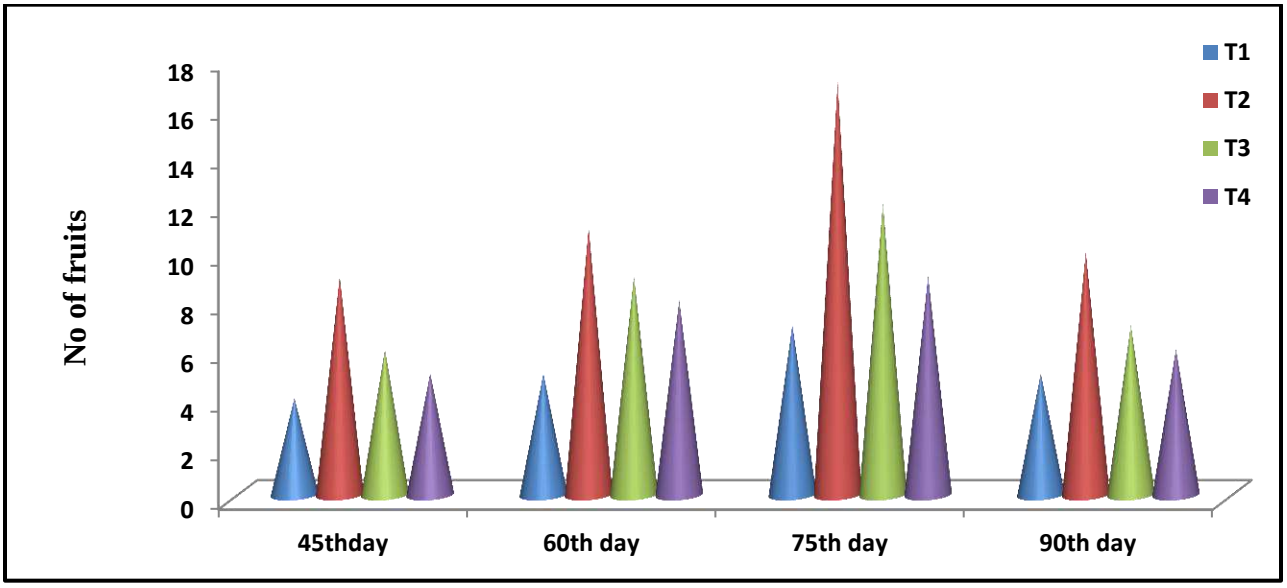
In general, it was observed that compost application significantly improved the overall plant growth of the tomato hybrid UNIK-38 under polyhouse conditions (Plate 4.14 to 4.18). Mango leaf litter composts, brinjal stalks composts and grape pruning's composts prepared using the Consortium-1 were able to improve the number of flowers, fruits and fruit yield of tomato hybrid UNIK-38. The increase in the number of flowers and fruits was observed across the day intervals (Figures 4.48 to 4.50; 4.57 to 4.62). *Dolichos* stover and chilli stalks composts prepared using the Consortium-2 were found to improve the numbers of flowers, fruits and the individual fruit weight of tomato hybrid UNIK- 38. The improvement in the flower and fruit numbers was observed across the day intervals (Figures 4.51 to 4.56).

Across substrate treatments it was observed that the composts prepared using Consortium -2 registered the highest dehydrogenase activities on the 45^{th} day after transplanting which coincides with the peak flowering stage of tomato (Figures 4.63 to 4.67).



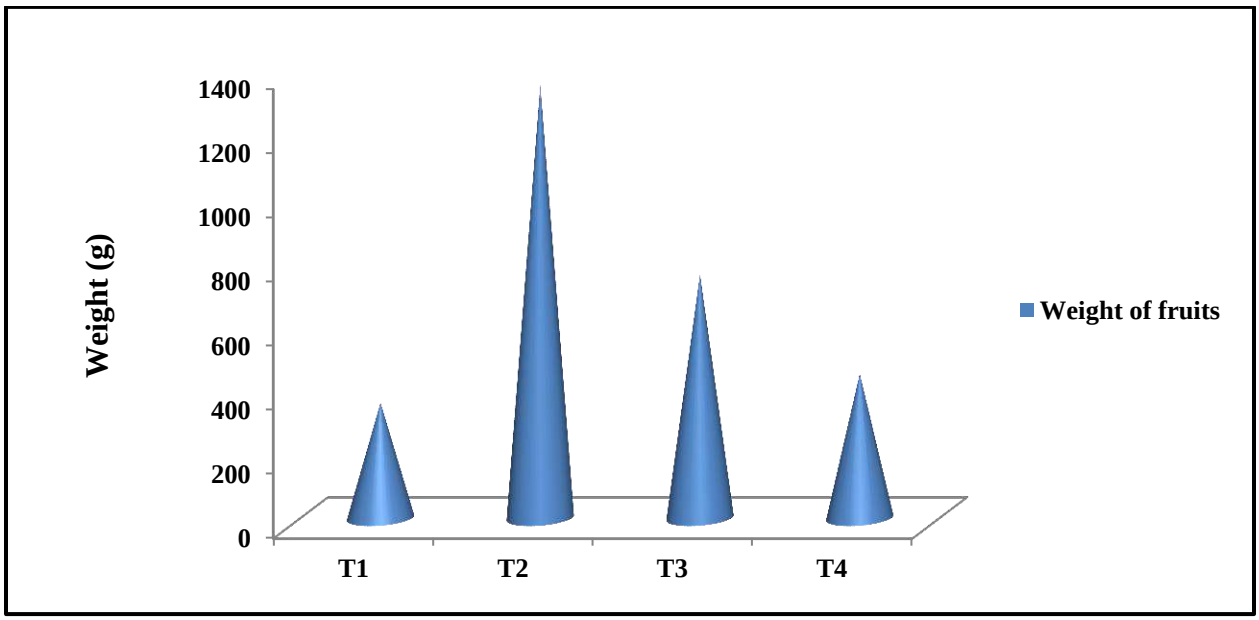
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.48. Effect of mango leaf litter compost on number of flowers of tomato



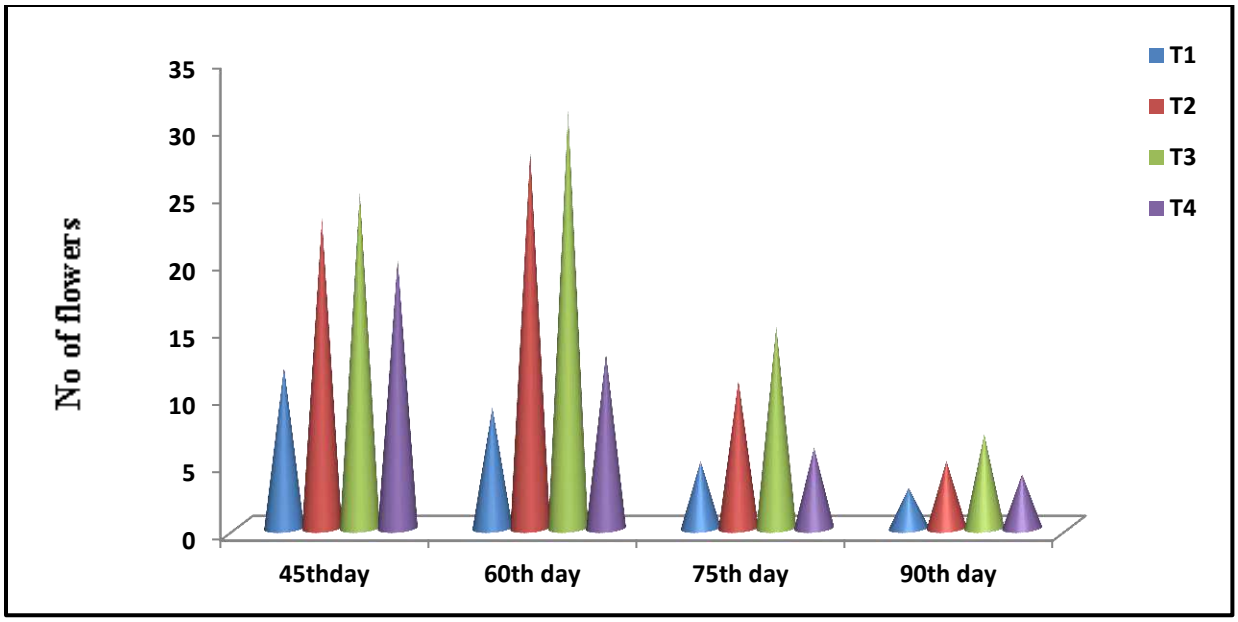
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.49. Effect of mango leaf litter compost on number of fruits of tomato



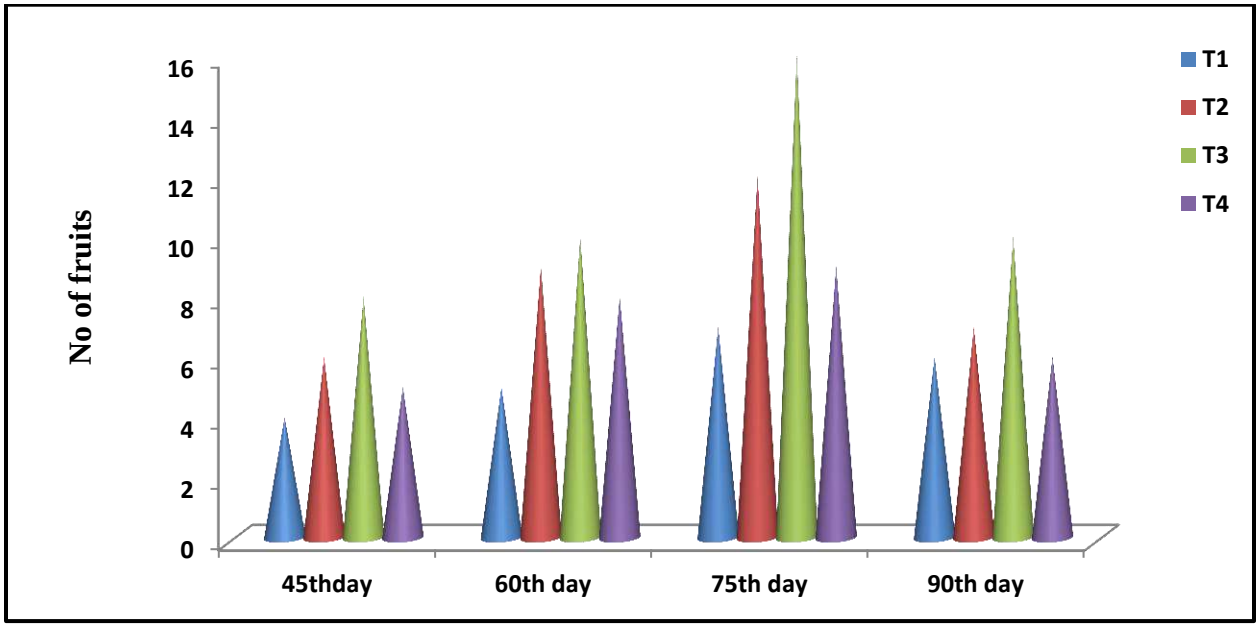
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.50. Effect of mango leaf litter compost on fruit weight of tomato



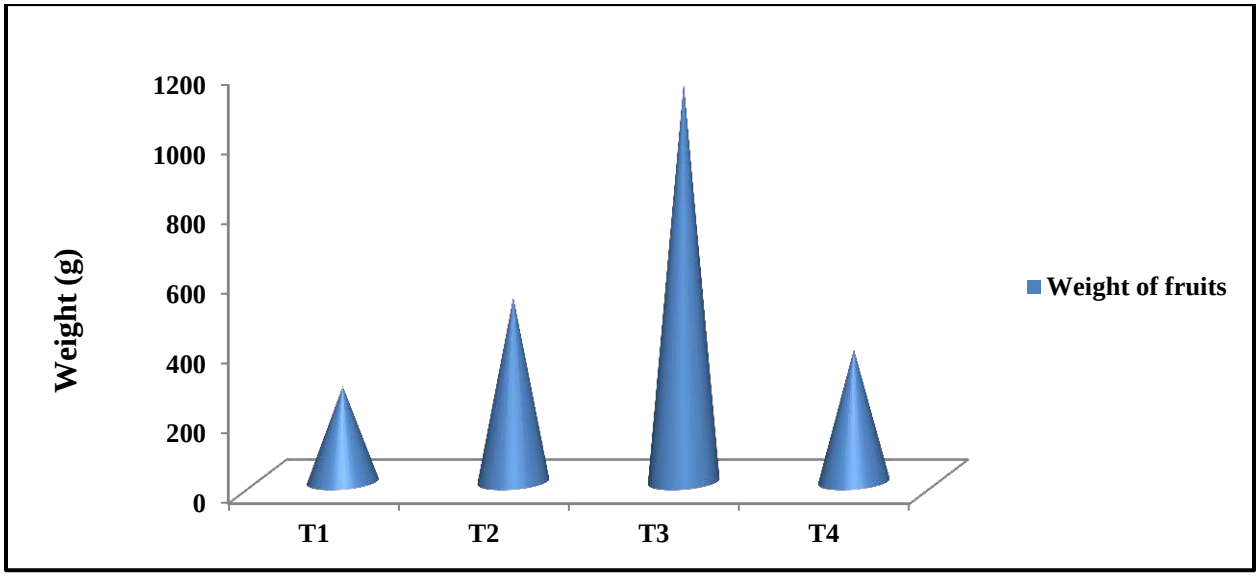
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.51. Effect of *Dolichos* stover compost on number of flowers of tomato



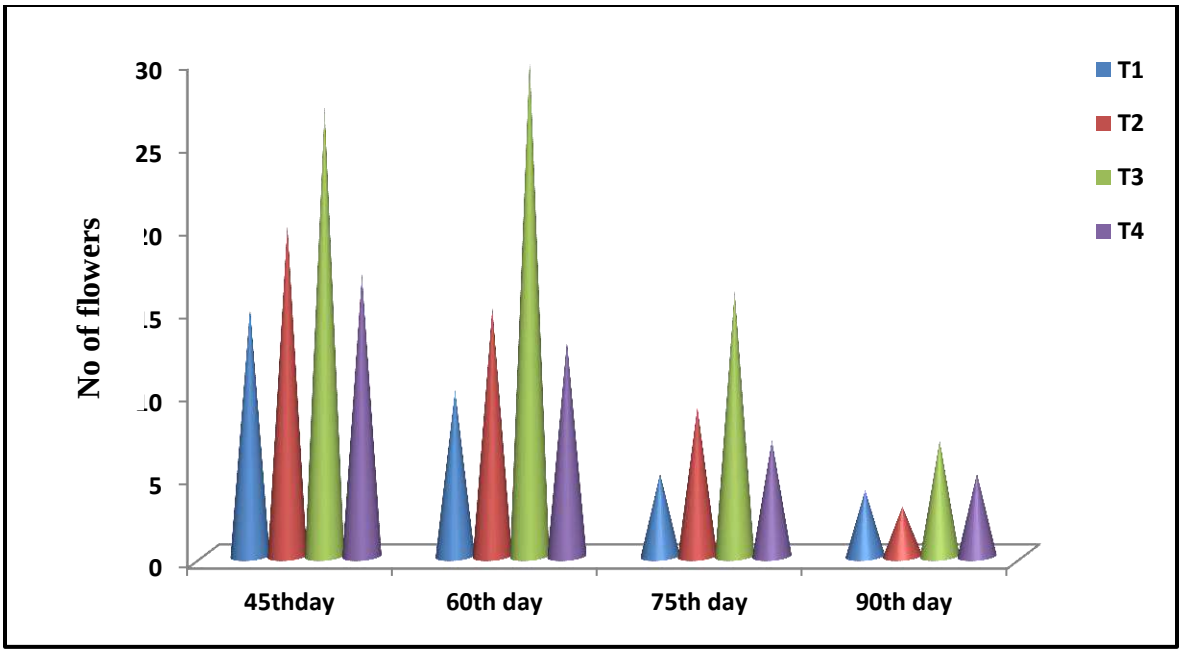
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.52. Effect of *Dolichos* stover compost on number of fruits of tomato



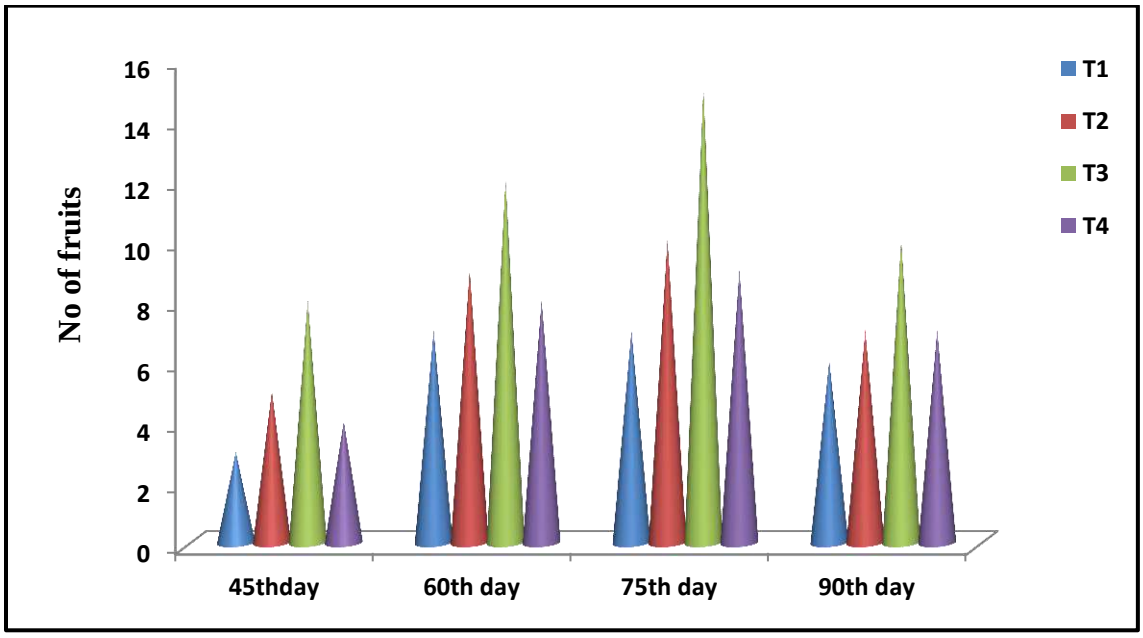
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.53. Effect of *Dolichos* stover compost on fruit weight of tomato



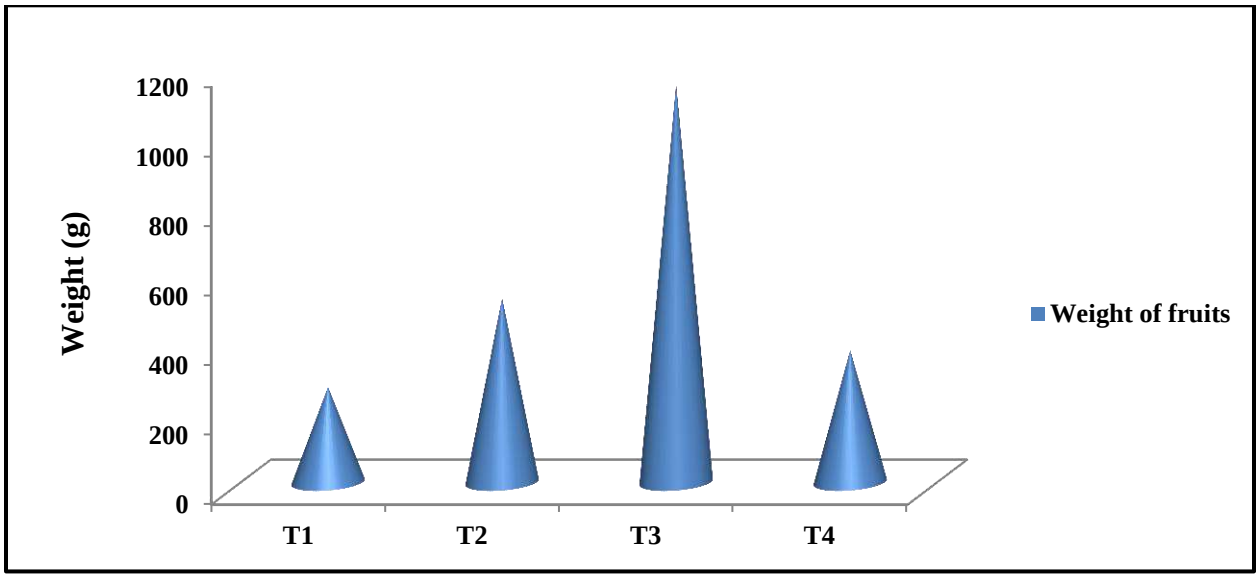
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.54. Effect of chilli stalks compost on number of flowers of tomato



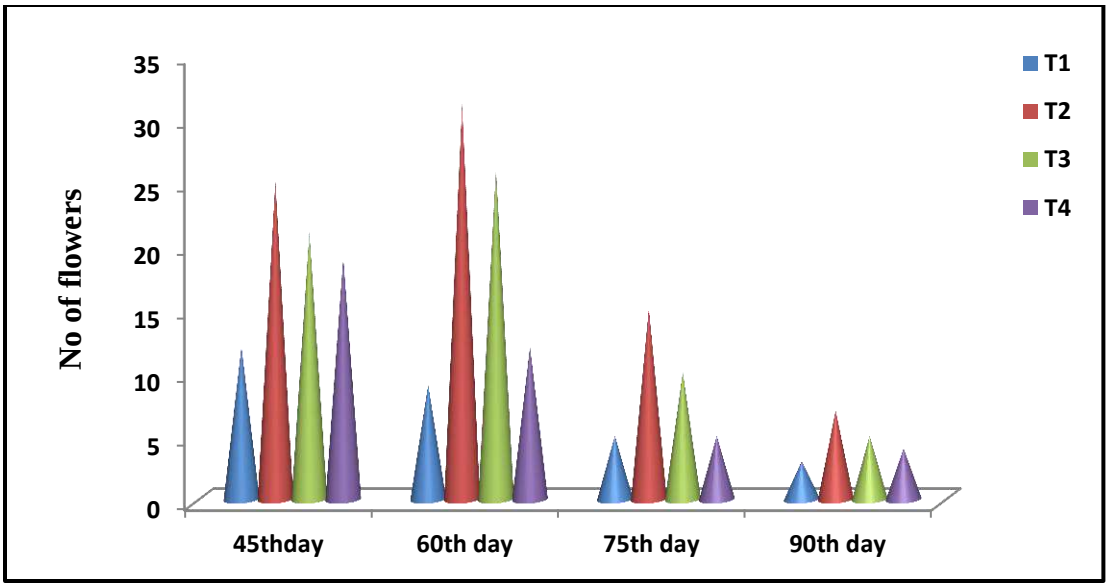
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.55. Effect of chilli stalks compost on number of fruits of tomato



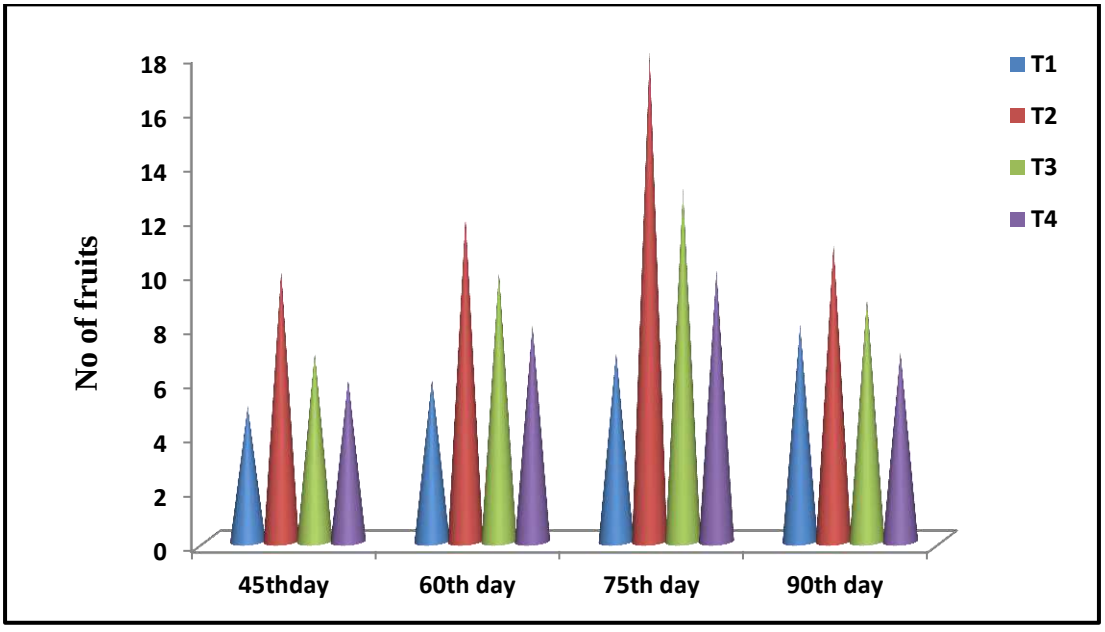
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.56. Effect of chilli stalks compost on fruit weight of tomato



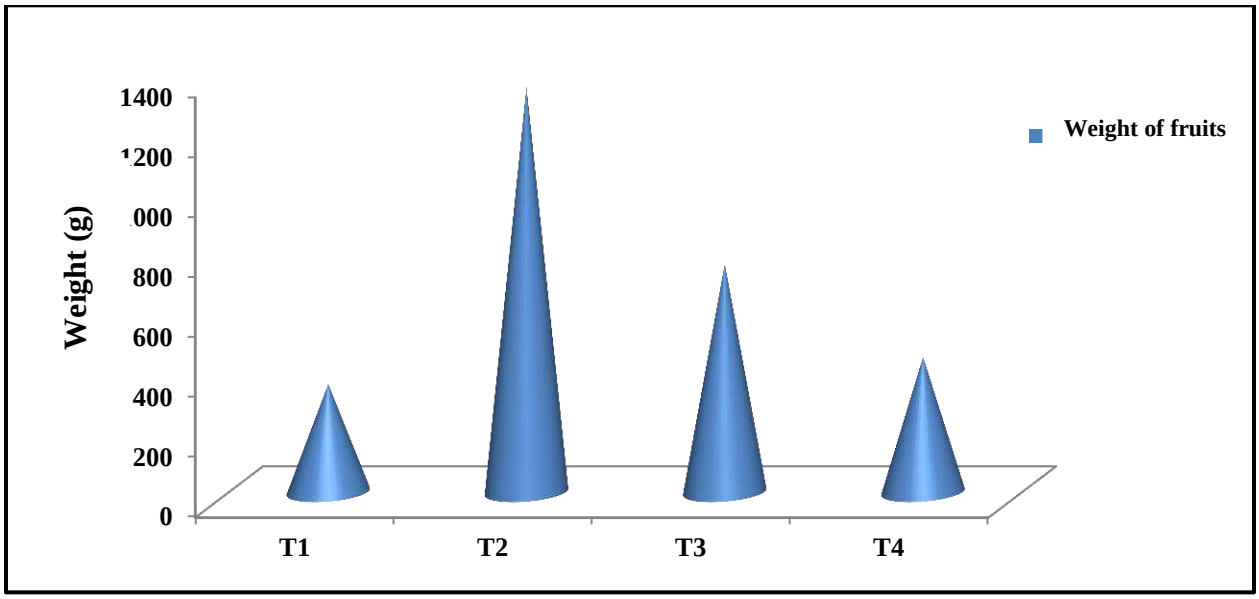
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.57. Effect of grape pruning's compost on number of flowers of tomato



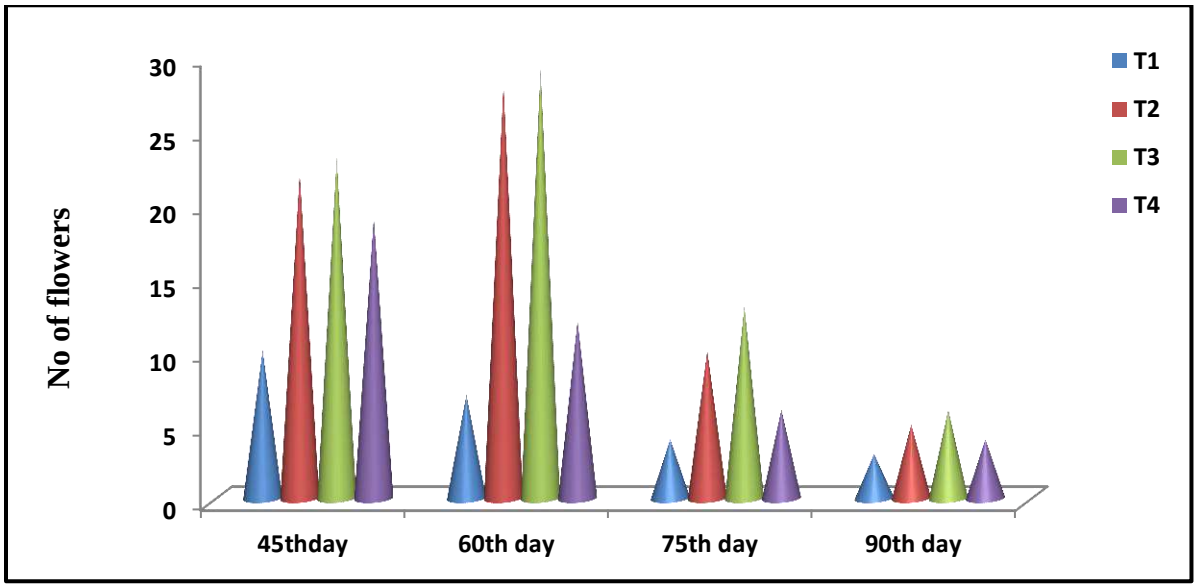
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.58. Effect of grape pruning's compost on number of fruits of tomato



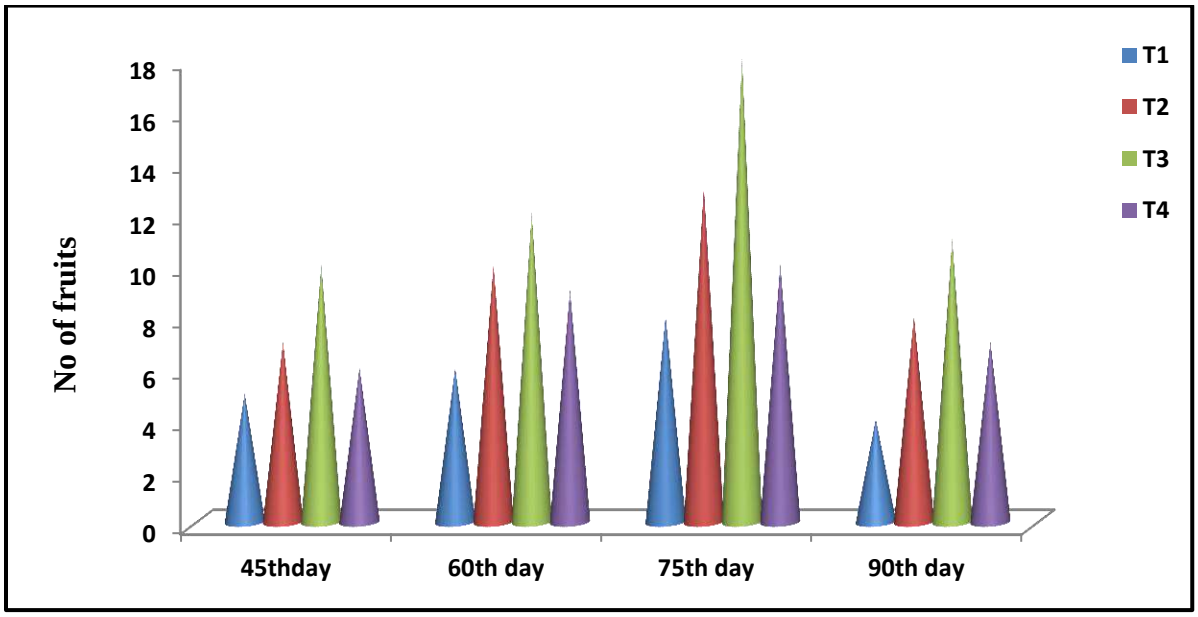
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.59. Effect of grape pruning's compost on fruit weight of tomato



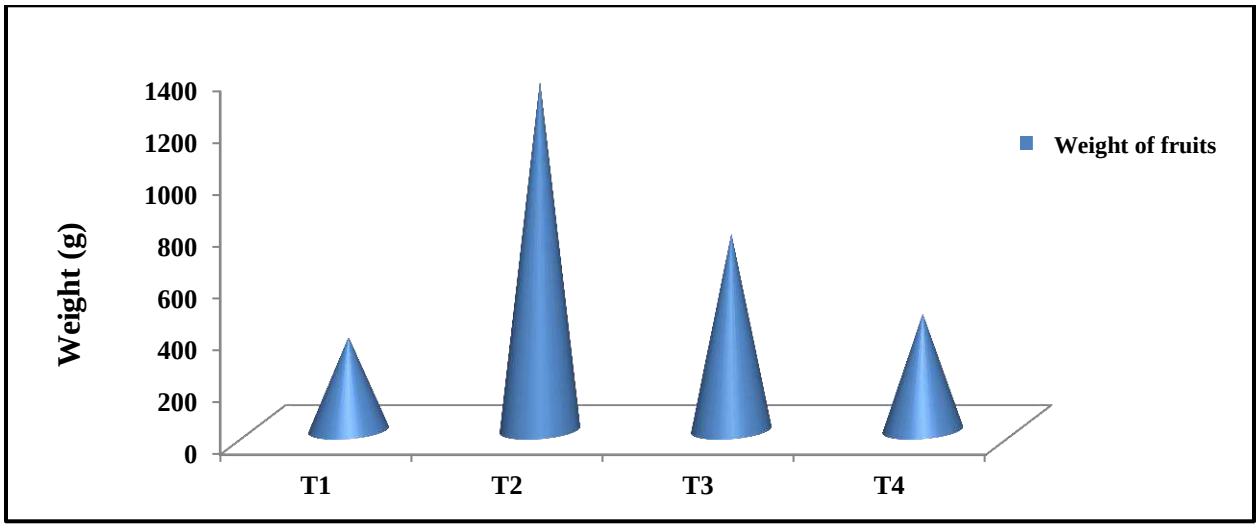
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.60. Effect of brinjal stalks compost on number of flowers of tomato



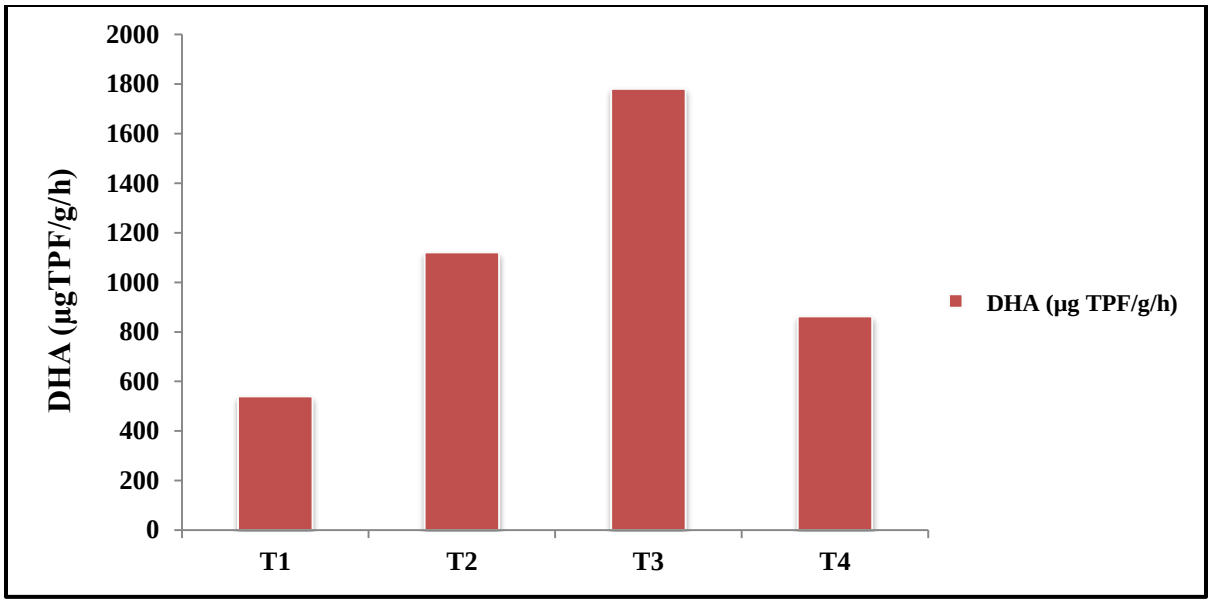
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.61. Effect of brinjal stalks compost on number of fruits of tomato



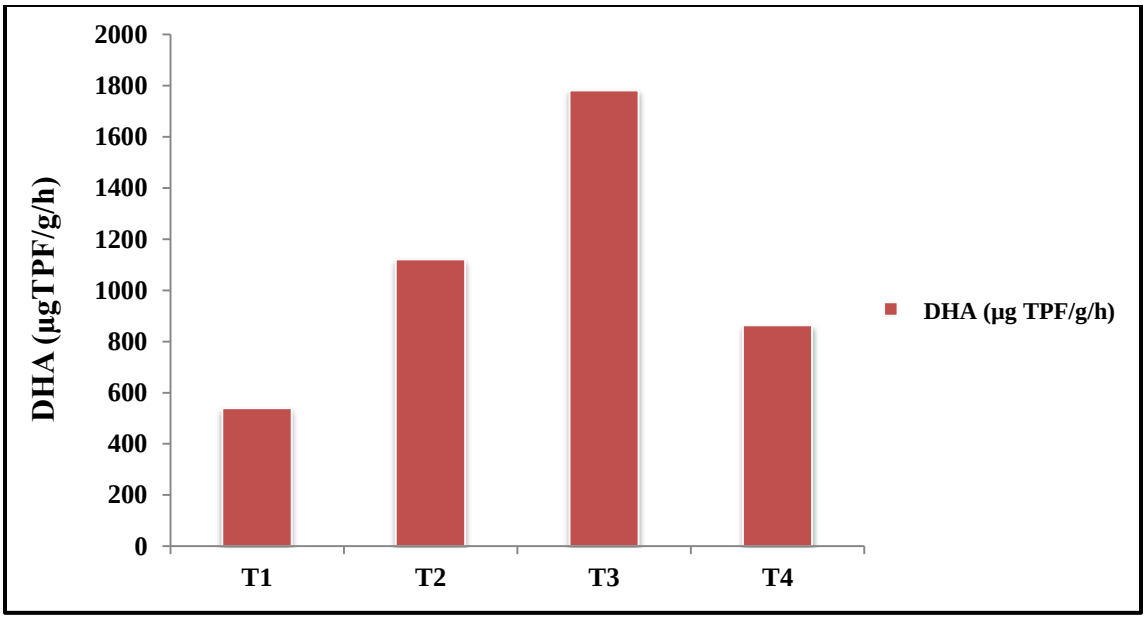
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.62. Effect of brinjal stalks compost on fruit weight of tomato



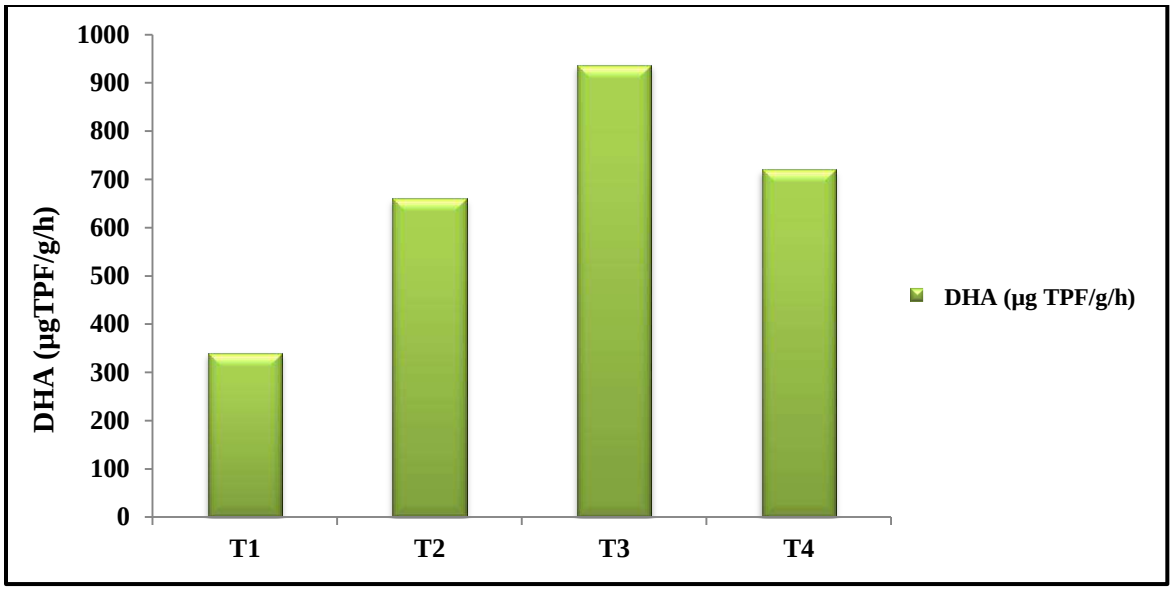
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.63. Dehydrogenase activity of mango leaf litter compost applied soils during the active flowering stage (45th day) of tomato.



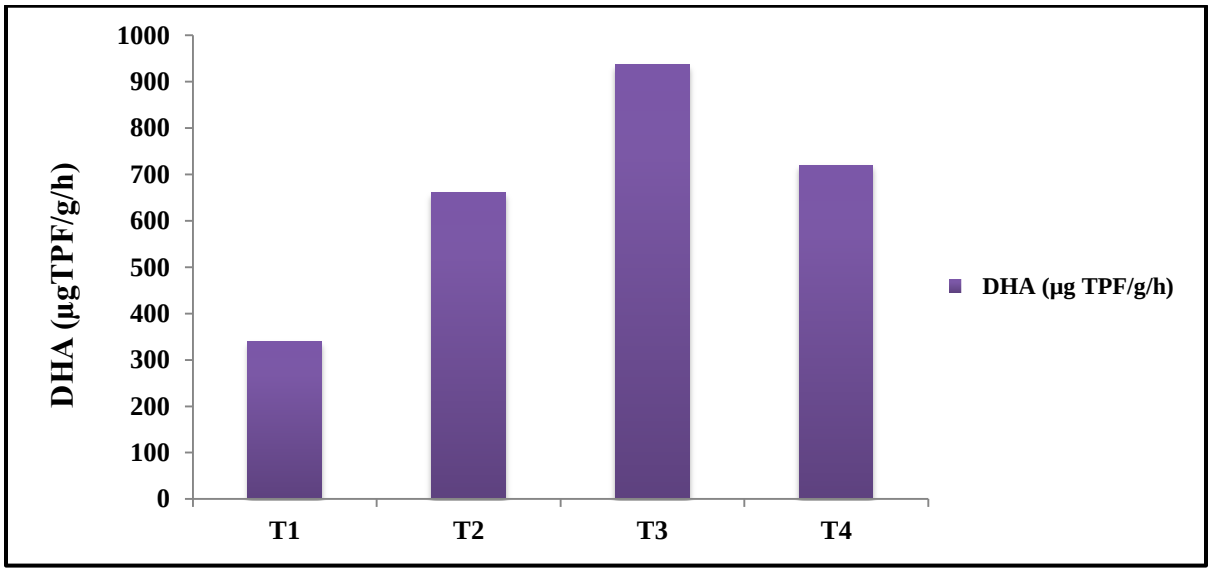
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.64. Dehydrogenase activity of *Dolichos* stover compost applied soils during the active flowering stage (45th day) of tomato



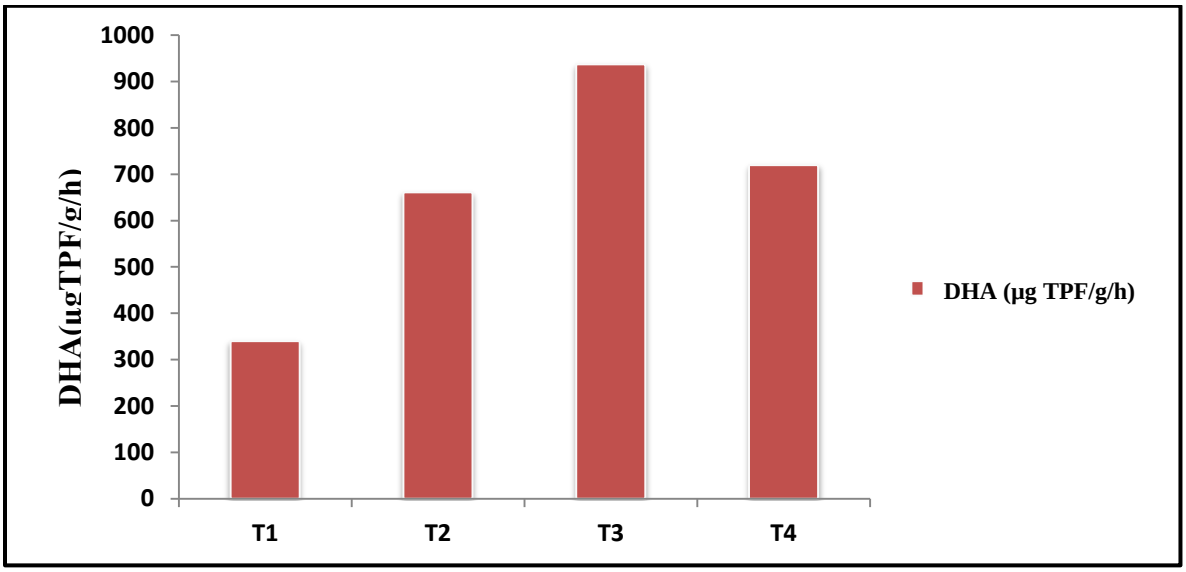
T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.65. Dehydrogenase activity of chilli stalks compost applied soils during the active flowering stage (45th day) of tomato



T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.66. Dehydrogenase activity of grape pruning's compost applied soils during the active flowering stage (45th day) of tomato



T1- Control; T2- Consortium 1; T3- Consortium 2; T4- Consortium 3

Figure 4.67. Dehydrogenase activity of brinjal stalks compost applied soils during the active flowering stage (45th day) of tomato

(A) 30th day of transplantation

(B) 45th day flowering stage

(C) 75th day fruiting stage

Plate 4.14. Evaluation of composts on tomato hybrid (UNIK 38) under polyhouse conditions

Plate 4.15. Evaluation of mango leaf litter compost on tomato hybrid (UNIK 38) under polyhouse conditions

Plate 4.16. Evaluation of *Dolichos* stover compost on tomato hybrid (UNIK 38) under polyhouse conditions

Plate 4.17. Evaluation of chilli stalks compost on harvest parameters of tomato hybrid (UNIK 38) under polyhouse conditions

Plate 4.18. Evaluation of brinjal stalk compost on harvest parameters of tomato hybrid (UNIK 38) under polyhouse conditions

CHAPTER-5

Discussion

DISCUSSION

In horticulture the generation of crop residues is enormous and composting of such residues helps in the replenishment of soil carbon and recycling of crop nutrients. Horticultural wastes are majorly composed of cellulose, hemicellulose, lignin, tannin and pectin. For the biodegradation of these residues, different microbial agents which produce hydrolytic enzymes are required as compost starters. In the present study different groups of potential microbial agents such as lignolytic, cellulolytic, hemicellulolytic, tannin degrading and pectinolytic microbes were isolated from samples such as compost yards, Farm Yard Manure (FYM) pits and perishable commodities disposal sites from periurban regions of Bengaluru. They were subjected to qualitative screening on selective media, which revealed that six cellulolytic bacterial isolates and four fungal isolates showed good cellulolytic activity on carboxy methyl cellulose agar (0.1% CMC) and Mandel Reese agar (1% CMC) respectively. The cellulolytic indices of the bacterial cultures *viz*., *Bacillus cereus* CB-7, *Bacillus* sp. CB-9, *Bacillus invictae* CB-16, *Bacillus tequilensis* CB-17, *Bacillus simplex* FLCB-5 and *Bacillus endophyticus* FLCB-11 ranged from 6- 8, whereas the fungal isolates *viz*., *Penicillium citrinum* CF-20, *Aspergillus* sp. TMLF-1, *Aspergillus niger* PMLF-1, *Aspergillus* sp. FCLF-1, had cellulolytic indices ranging from 1-1.6.

This observation was supported by the findings of Uttam Kumar *et al*. (2014), who reported that *Trichoderma viride, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Fusarium oxysporum, Trichoderma* sp., *Curvularia* sp., *Penicillium* sp., *Alternaria* sp. and *Rhizopus* sp. were common cellulase producing fungi isolated from forest litters and *Trichoderma viride* and *Aspergillus niger* were efficient cellulase producers on Carboxy Methyl Cellulose (CMC) agar.

Jahangeer *et al*. (2005), reported that cellulolytic fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Penicillium* sp., *Alternaria* sp., *Rhizhopus* sp., *Trichoderma harzanium and Mucor* sp. isolated on modified Mandel's medium from environmental samples such as soil and air exhibited cellulase activity with clear zones ranging from 1-18 mm. Similarly Devi *et al*. (2012) isolated fungi *viz.*, *Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp. and *Alternaria* sp. that showed appreciable cellulolytic activity on CMC (1%) agar and xylanolytic activity on medium supplemented with birchwood xylan. The zone of clearance on CMC agar was found to be 13-40 mm and 16-38 mm on xylan agar respectively.

Tannin is an important compound present in the plant system that greatly retards microbial activity. Tannase producing fungi are able to degrade the tannin and hence play an important role in the bioconversion of tannin rich substrates. In the present study the tannase producing fungal isolates *viz*., *Aspergillus fumigatus* CF-17, *Penicillium chrysogenum* VCLF-1 and *Aspergillus sp*. TL-8 were found to possess tannase indices ranging from 2-3.5. This finds support from the earlier findings of Brahmbhatt *et al*. (2014) that tannase producing fungi were widely distributed amongst the genera *Penicillium, Aspergillus, Mucor* and *Rhizopus.* Girdhari *et al*. (2015) have also reported the screening of tannase producing fungal species of *Penicillium citrinum, Aspergillus fumigatus and Penicillium lividum*. Owing to biosafety concerns the isolate *A. fumigatus* CF-17 was not utilized in compost production studies.

In the present work the pectinolytic activity of fungi was screened on Mineral Salt Agar containing (1%) pectin. The isolates *Penicillium chrysogenum* VCLF-1, *Aspergillus affustus* FLF-11 and *Alternaria tenuissima* LG-1 showed pectinolytic indices ranging from 1.7-2. Similar results were obtained by Priya and Sashi (2014) who isolated and screened pectinolytic fungi

such as *Aspergillus niger*, *Aspergillus versicolor*, *Aspergillus flavus*, *Fusarium oxysporum*, *Penicillum citrinum*, *Trichoderma viride* and *Mucor* sp. from pectin rich sites on Czapek-Dox agar containing 1.5% carbon pectin. These isolates showed pectinolytic activity in the form of clearance zone ranging from 1-4 mm around the colonies. Sandhya and Kurup (2013) also found that *Penicillium citrinum* is a potent pectinase producer.

In the present study the laccase producing abilities of the fungi was determined on lignin basal medium with 0.1% (w/v) ABTS (2-2'-azino-bis (3-ehylbenz-thiazoline-6-sulfonic acid). This finding was supported by Singh *et al*. (2013) who reported the detection of laccase producing *Fusarium* sp. and *Aspergillus* sp. on Potato Dextrose Agar incorporated with the indicator compound ABTS. But none of isolates used in this study were able to exhibit peroxidase activities.

In the present work the quantification of cellulase activity by the Filter Paper Assay (FPA) revealed appreciable total cellulase activity of 0.0169 IU/ml by the isolate *Bacillus tequilensis* CB-17, while the fungal isolate *Aspergillus* sp. FCLF-1 possessed the highest total cellulase activity of 0.049 IU/ml. Jahangeer *et al*. (2015) reported that the cellulase activity of *Aspergillus niger* was found to be 0.123 IU/ml, while *Trichoderma harzanium* and *Trichoderma longibrachiatum* showed cellulase activities of 0.034 IU/ml and 0.059 IU/ml respectively. Amongst the hemicellulolytic enzymes, xylanase is commonly used as an indicator of an organism's hemicellulolytic activities. In the present study the xylanolytic activity was highest in the case of the isolate *Aspergillus* sp. CF-11 (0.09 µmol/ml/min). This finds support from Karunakaran *et al*. (2014) who reported that *Aspergillus niger* was a powerful xylanase producer.

In the present study *Penicillium chrysogenum* VCLF-1 showed good tannase activity as determined by the quantity of gallic acid produced by HPLC analysis. Similar results were obtained by Srivastava *et al*. (2009), that submerged fermentation was suitable for tannase and gallic acid production from *Aspergillus niger*. Murgan *et al*. (2007) also reported the production of tannase from *Aspergillus niger* MS101 by submerged fermentation and stated that *Aspergillus* sp.were very good tannase producers which is in accordance with the present study.

In the present study the fungal isolate *Alternaria tenuissima* LG-1 had a pectinase activity of a 2.511 µmol/ml/min followed by *Penicillium chrysogenum* VCLF-1 and *Aspergillus affustus* FLF-11. Sandhya and Kurup (2013) reported that the pectinolytic activity of *Penicillium citrinum* was found to be 0.067 µmol/ml/min. Similarly Bankar *et al*. (2012) reported a pectinase activity of 5.38 U/mL in *Mortiella* sp. isolated from forest soils of Bhadra wild life sanctuary in Western Ghats of Southern India.

The determination of the humification potential can serve as an early tool for the rapid screening of isolates since it provides an insight in to the ability of the organisms. Haseena *et al*. (2016) have recommended the screening of organisms on two different wastes. In the present study the *in vitro* composting studies using chilli stalks as substrate revealed that most of the elite isolates possessed good humification potential.

Strain compatibility is an important determinant in mixed cultures, which influences distribution, organization, ecological balance of communities and density of microhabitat population. In the present study the compatibility between different groups of elite isolates revealed that most of the elite isolates were compatible with each other, while a few isolates were found non-compatible. Kausar *et al*. (2010) reported the compatibility and mutual

intermingling of lignocellulolytic fungi that were used in consortium development for the biodegradation of rice straw. This is a crucial aspect that determines the success of the developed consortia.

In the present study consortium used for composting process contains mixed cultures of bacteria and fungi. Gutierrez- Correa and Tengerdy (1997); Molla *et al*. (2001) reported fungi can invade the substrate quickly because of filamentous nature and their ability to produce prolific spores. Fungi play a major role in composting of lignocellulosic waste. Game *et al*. (2017) reported the decomposition of organic matter is enhanced by the presence of cellulolytic microorganisms in consortium and aids in conversion of organic matter into nutrient rich compost in short time. In the present study lignite was used as a carrier material for consortia development. This finds support in the observations of Chandran *et al*. (2014) reported that lignite can be used as carrier material in consortium preparation for degradation of lignocellulolytic waste.

Stability and maturity are two of the most important factors which may affect the agronomic efficiency of composts. Stability is defined as the degree of stabilization of the organic matter present in the composts and is inversely related to the microbial activity and the bioavailability of organic matter. Maturity refers to the degree of decomposition of phytotoxic substances and the adequacy of the compost for plant growth (Butler *et al*. 2001; Benito *et al*. 2003; Said – Pullicino *et al*. 2007). In the present study three consortia consisting of different groups of microbes were evaluated under controlled conditions for the bioconversion of horticultural residues in to compost for different time periods based on the nature of the substrate. The composts obtained were analyzed for their stability and maturity parameters *viz*., Carbon: Nitrogen (C:N) ratio, humification index, moisture content, contents of Total Organic

Matter (TOM), Total Organic Carbon (TOC), Water Soluble Carbon (WSC), Water Soluble Nitrogen (WSN), Humic like Carbon (HC), Fulvic like Carbon (FC), Alkali Extractable Carbon (C_{ext}), phytotoxicity assay and Germination Index (GI) of radish seeds.

In the present work, the pH of prepared compost samples was around 7.0-7.7. Ieshita *et al.* (2013) reported that the pH of wheat straw compost was around 7 and the alkaline pH range is preferred in the final composts. It has been reported that the pH of the final compost ranges from 7-8 as organic matter decomposes gradually with time, stabilizes with final pH of compost between 7 to 8 with a concomitant reduction in the C: N ratio (Gade *et al*. 2010; Himanen and Hanninen, 2011; Raj and Antil, 2011; Ranalli *et al*. 2001 and Sarker *et al.* 2013). Nakasaki *et al*. (2011) reported that in pH range of 7 to 8, the microbes show the highest degradation activity.

In the present work the moisture content of composts were maintained at a constant level during initial stages of composting and gradually reduced to 30-40% at the end of composting. Epstein *et al*. (1997) reported that at the end of composting, the reduced moisture content of composts is a positive sign of decomposition and maturity of the compost, which is in support of the present study.

The Carbon to Nitrogen (C: N) ratio is an important parameter that determines the extent of decomposability of a substrate. A C: N ratio of 20:1 is usually an acceptable parameter for good quality composts (Iglesias and Perez 1989). In the present work the Carbon: Nitrogen (C: N) ratio of mango leaf litter was reduced from 43.48 to 15.5 after 90 days of composting by Consortium-1, the C: N ratio of *Dolichos* stover reduced from 45 to 14.8 after 90 days of composting by Consortium-2, the C: N ratio of chilli stalks reduced from 80 to 16.13 after 150 days of composting by Consortium-2, the C: N ratio of grape pruning's was reduced from 60 to

14.4 after 150 days of composting by Consortium-1 and the C: N ratio of brinjal stalks reduced from 55 to 15.60 by Consortium-2. Goyal *et al*. (2011) reported that during composting of rice straw by different inocula, the C: N ratio reduced from 73.7-16.6. Haseena *et al*. (2016) reported that during composting of weed wastes, ayurvedic herbal wastes, coir pith wastes and sawdust the C: N ratio showed significant reduction in final compost samples.

The Water Soluble Carbon (WSC) content of the prepared composts is an important parameter that has been related to maturity by many authors as being a direct measure of the water soluble fraction of organic matter which is most accessible to microorganisms during composting (Zmora- Nahum *et al*. 2005; Said Pullicino *et al*. 2007). In this study, mango leaf litter compost and grape pruning's compost produced using Consortium-1 possessed WSC contents of 3.5 g/kg and 5.1 g/kg respectively. The *Dolichos* stover composts, chilli stalks compost and brinjal stalks composts produced using Consortium-2 had WSC content of 2.4 g/kg, 3.2 g/kg and 3.6 g/kg respectively. These observations are in concurrence with Paradelo *et al*. (2013) who reported that WSC of less than 5 g/kg is a good indicator of compost stability. The WSC/N ratio is also a good indicator of maturity (Garcia *et al*. 1992; Pascual *et al*. 1997). A WSC/N ratio below 0.5 is considered a good indicator of compost maturity.

The Humic like Carbon (HC)/ Fulvic like Carbon (FC) ratios of the mango leaf litter and grape pruning's composts prepared using Consortium-1 was 3.3 and 4.63 respectively. While the HC/FC ratio of the *Dolichos* stover compost, chilli stalks compost and brinjal stalks composts prepared using Consortium-2 were 3.13, 6.09 and 4.63 respectively. It has been reported that the ratio of HC/FC would increase during composting and this ratio is the most representative of the state of humification (Iglesias and Perez, 1992). Paradelo *et al*. (2013) reported that the HC/FC

ratio of the final compost greater than 1.9 is a good indicator of humification. The results of this present study are in accordance with the earlier findings.

The $Q_{4/6}$ ratio is an indirect measure of the humification and is commonly referred to as the humification index. A $Q_{4/6}$ ratio lower than 5.0 is accepted as a good indicator of compost maturity (Chen *et al*. 1997). In the present study most composts recorded a $Q_{4/6}$ ratio lower than 5.0 with the exception of the brinjal stalks compost which had a $Q_{4/6}$ ratio of 6.4. The lack of a trend between the $Q_{4/6}$ and HC/FC ratio has been reported by Znora-Nahum *et al*. (2005) who proposed the use of absorption at 445 nm as an indirect measure of compost maturity.

In the present study compost maturity was evaluated by recording the percent germination and Germination Index (GI) of radish *Raphnus sativus* seeds. Selim *et al*. (2012) reported that germination index and seed germination (%) was increased with composting progresses. NH_4^+-N and heavy metal concentration was decreased in final compost samples which were main inhibitors of seed germination and root elongation. Mango leaf litter and grape pruning's composts prepared by using Consortium-1 recorded percent germination of 90 and germination indices of 371.58 and 431.51 respectively. The *Dolichos* stover, chilli stalks and brinjal stalks composts prepared using Consortium-2 recorded percent germination of 90 and germination indices of 224, 308.2 and 308.21 respectively. According to Zucconi et al. (1985) the percent germination values below 50% mean high phytoxicity and values between 50 to 80% represent medium phytotoxicity and values over 80% represent no phytotoxicity. By this scale it is apparent that the composts produced in this study do not have any phytoxicity.

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CHAPTER-6

Summary

SUMMARY

Composting is the process of controlled microbial aerobic decomposition and stabilization of organic substrates to produce a stable end product. The term compost refers to the stable, humus like product resulting from the biological decomposition of organic matter under controlled conditions. In horticulture, the generation of crop residues is enormous and composting of such residues helps in replenishment of soil carbon and recycling of crop nutrients. For an efficient composting process, microbial consortia can be used as starters. As horticultural residues are majorly composed of cellulose, hemicelluloses, tannin, pectin and lignin, any formulated consortium should contain different groups of microbes such as cellulolytic, hemicellulolytic, tannin degrading and lignolytic in order to attain the desirable level of composting.

Seventy fungal and twenty-five bacterial cultures isolated from samples originating from compost yards, Farm Yard Manure (FYM) pits and perishable commodities disposal sites from periurban regions of Bengaluru were screened for their enzymatic activities qualitatively and quantitatively and the elite isolates were obtained and identified through molecular characterization. Based on the compatibility amongst the isolates three consortia were formulated and evaluated for their ability to produce compost using succulent, semi-succulent and woody horticultural residues. The mango leaf litter and *Dolichos* stover residues could be composted in 90 days while chilli stalks, brinjal stalks and grape pruning's were composted in 150 days. The prepared composts were analyzed for different maturity and stability parameters, which were significantly better than the inoculated controls. The Consortium -1 comprising (*Aspergillus sp.* TMLF-1, *Aspergillus oryzae* CP-2, *Trichoderma hamatum* FLF-13, *Aspergillus sp.* TL-8) was found suitable for the composting of mango leaf litter and grape pruning's, while

the Consortium 2 comprising (*Bacillus endophyticus* FLCB-11, *Aspergillus sp*. CF-11, *Xylaria* sp. TF-4, *Penicillium chrysogenum* VCLF-1) was found suitable for the composting of *Dolichos* stover, chili and brinjal stalks. When composts were evaluated on tomato under polyhouse conditions, it was observed that the composts significantly improved the vegetative and harvest parameters of tomato under polyhouse conditions. Based on the overall performance of the starter consortia it is concluded that the Consortium -2 is more suited for the bioconversion of a wide range of horticultural crop residues ranging from succulent to woody, while Consortium -1 is more suited for the bioconversion of mango leaf litter and grape pruning's.

Conclusion

CONCLUSION

Horticultural residues being multifarious in nature and ranging from succulent to woody in composition require specialized microbial consortia for their bioconversion into composts. In the present study after intensive screening and selection three consortia were formulated for composting trials using horticultural crop residues. The Consortium -1 comprising (*Aspergillus sp.* TMLF-1, *Aspergillus oryzae* CP-2, *Trichoderma hamatum* FLF-13, *Aspergillus sp.* TL-8) was found suitable for the composting of mango leaf litter and grape pruning, while the Consortium -2 comprising (*Bacillus endophyticus* FLCB-11, *Aspergillus sp*. CF-11, *Xylaria* sp. TF-4, *Penicillium chrysogenum* VCLF-1) was found suitable for the composting of *Dolichos* stover, chili and brinjal stalks. This reinforces the fact for the need for a specialized consortium for the bioconversion of horticultural crop residues. When the prepared composts were evaluated on tomato under polyhouse conditions, it was observed that through all the prepared composts significantly improved the vegetative and harvest parameters of tomato under polyhouse conditions, the composts prepared using Consortium -2 were significantly superior in improving vegetative and harvest parameters of tomato crop, besides improving the soil microbial activity as evidenced by the higher dehydrogenase activity, recorded during the peak flowering stage of tomato.

References

REFERENCES

1. Adesina, F. C. and Onilude, A. A. (2013). Isolation, identification and screening of xylanase and glucanase producing microfungi from degrading wood in Nigeria. *African Journal of Agricultural Research*, 8: 4414-4421.
2. Ahmad, M., Taylor, C. R., Pink, D., Burton, K., Eastwood, D., Bending, G. R. and Bugg, T. D. H. (2010). Comparative analysis of bacterial and fungal lignin degraders. *Molecular Biosystems*, 6: 815-821.
3. Alexander, M. (1961). Microbiology of cellulose. In: Introduction to Soil Microbiology (2^{nd} ed.). John Wiley and Son, Inc. New York and London.
4. Alfarra, H. Y., Hasali, N. H. M. and Omar, M. N. (2013). Lignolytic fungi with laccase activity isolated from Malaysian local environment for phytochemical transformation purposes. *International Research Journal of Biological Sciences*, 2: 51-54.
5. Annamalai, N., Thavasi, J. S. and Balasubramanian. (2009). Thermostable and alkaline tolerant xylanase production by *Bacillus subtilis* isolated from marine environment. *Indian Journal of Biotechnology*, 8: 291-297.
6. Aoki, K., Shinke, R. and Nishira, H. (1976). Chemical composition and molecular weight of yeast tannase. *Agricultural Biological Chemistry*, 40: 297–302.
7. Bailey, M. J., Biley, P. and Poutanen, K. (1992). Interlaboratory testing methods for assay of xylanase activity. *Journal of Biotechnology*, 23: 257-270.
8. Bajpai, B., Banerjee, T. and Patil, S. (1999). Gallotannin hydrolysis by immobilized fungal mycelia in a packed bed bioreactor. *Indian Journal of Experimental Biology*, 37: 94-97.

9. Balajee, S. A., Houbraken, J., Verweij, P. E., Hong, S. B., Yaghuchi, T., Varga, J. and Samson, R. A. (2007). *Aspergillus* species identification in the clinical setting. *Studies in Mycology*, 59: 39-46.

10. Banakar, S. P. and Thippeswamy, B. (2012). Isolation, production and partial purification of fungal extracellular pectinolytic enzymes from the forest soils of Bhadra Wildlife Sanctuary, Western Ghats of Southern India. *Journal of Biochemical Technology*, 3: 138-143.

11. Barbosa, A. M., Dekker, R. F. H. and Hardy, G. E. (1996). Veratryl alcohol as an inducer of laccase by an ascomycete, *Botryosphaeria* sp, when screened on polymeric dye Poly R-478. *Letters in Applied Microbiology*, 23: 93-96.

12. Bazrafshan, E., Zarei, A., Mostafapour, F. D., Poormollae, N., Mahmoodi, S. and Zazouli, M. A. (2016). Maturity and stability evaluation of composted municipal solid wastes. *Health Scope*, 5: 1-9.

13. Beguin, P. and Aubert, J. P. (1994). The biological degradation of cellulose. *FEMS Microbiology Reviews*, 13: 25-28.

14. Benito, M., Masaguer, A., Moliner, A., Arrigo, N. Palma, R. M. (2003). Chemical and microbiological parameters for the characterization of the stability and maturity of pruning waste compost. *Biology and Fertility of Soils*, 37: 184-189.

15. Bertolin, T. E., Costa, J. A. V., Pasquali, G. D. L. (2001). Glucoamylase production in batch and fed batch solid-state fermentation: effect of maltose or starch addition. *Journal of Microbiology and Biotechnology*, 11: 13-16.

16. Bobbie, J. and Leatherwood, J. M. (1976). Derepressed synthesis of cellulose by *Cellulomonas*. *Journal of Bacteriology*, 128: 609-615.

17. Bradoo, S., Gupta, R. and Saxena, R. K (1997). Parametric optimization and biochemical regulation of extracellular tannase from *Aspergillus japonicus*, *Process Biochemistry,* 32: 135-139.

18. Brahmbhatt, D., Modi, H. A. and Jain, N. K. (2014). Preliminary isolation and screening of tannase producing bacteria and fungi. *International Journal of Current Microbiology and Applied Sciences,* 3: 193-203.

19. Bremner, J. M. (1996). Chapter 37 –Nitrogen –Total: In Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Series no. 5.

20. Burlacu, A., Cornea, C. P. and Israel-Roming, F. (2016). Microbial xylanase: a review. *Scientific Bulletin Series F.Biotechnologies*, 20: 335-342.

21. Butler, T. A., Sikora, L. J., Steinhilber, P. M., Douglass, L. W. and Sikora, L. J. (2001). Compost age and sample storage effects on maturity indicators of biosolids compost. *Journal of Environmental Quality*, 30: 2141-2148.

22. Carmen, V. G., Francisca, S. E., Lopez, M. J. and Moreno, J. (2006). Influence of microbial inoculation and co-composting material on the evolution of humic like substances during composting of horticultural wastes. *Process Biochemistry*, 41: 1438-1443.

23. Cavalitto, S. F., Arcas, J. A. and Hours, R. A. (1996). Pectinase production profile of *Aspergillus foedidus* in solid state cultures at different acidities. *Biotechnology Letters,* 18: 251-256.

24. Chandran, M., Manisha, A. and Subashini, A. (2014). Production of phosphate bio fertilizer using lignocellulosic waste as carrier material. *Asian Journal of Chemistry*, 26: 2065.

25. Charita Devi, M. and Kumar, S. M. (2012). Isolation and screening of lignocellulose hydrolytic saprophytic fungi from dairy manure soil. *Annals of Biological Research*, 3: 1145-1152.

26. Chen, S., Coffin, D. E. and Malone, R. F. (1997). Sludge production and management for recirculating aqua cultural systems. *Journal of World Aquaculture Society*, 28: 303-315.

27. Christie, S. A. D. and Shanmugam, S. (2012). Analysis of fungal cultures isolated from Anamalai hills for laccase enzyme production, effect on dye decolourization, antimicrobial activity. *International Journal of Plant, Animal and Environmental Science*, 2: 143-148.

28. Crawford, D. L., Sutherland, T. B., Pommeto, A. L. and Miller, J. M. (1982). Production of aromatic aldehyde oxidase by *Streptomyces viridosporus*. *Archives of Microbiology,* 131: 351-355.

29. Deepanjali, L., Divya, S., Verma, H. N. and Joy, J. G. (2012). Production, characterization and purification of tannase from *Aspergillus niger*. *Journal of Microbiology and Biotechnology Research*, 2: 566-572.

30. Dennis, C. and Webster, J. (1971). Antagonistic properties of species group of *Trichoderma* II production of non-volatile antibiotics. *Transactions of the British Mycological Society*, 57: 41-48.

31. Desai, S. S., Tennali, G. B., Channur, N., Anup, A. C., Deshpande, G. and Murtuza B.P.A. (2011). Isolation of laccase producing fungi and partial characterization of laccase. *Biotechnology, Bioinformatics, Bioengineering*, 1: 543-549.

32. Deschamps, A. M., Otuk, G. and Lebeault, J. M. (1983). Production of tannase and biodegradation of chesnut tannin by bacteria. *Journal of Fermentation Technology*, 61: 55-59.

33. Dickson, N., Richard, T. and Kozlowski, R. (1991). Composting to reduce the waste stream: A guide to small scale food and yard waste composting. Ithaca, NY: Natural Resource, Agriculture and Engineering Service Cooperative.

34. Ducros, V., Brzozowski, A. M., Wilson, K. S., Brown, S. H., Ostergaard, P., Schneider, P., Yaver, D. S., Pedersen, A. H .and Davies, G. J. (1998). Crystal structure of the type-2 Cu depleted laccase from *Coprinus cinereus*. *Nature Structural Biology.* 5, 310–316.

35. Epstein, E. (1997). The science of composting. Technomic Publishing Company, Lancaster, PA.

36. Favela-Torres, E., Volke-Sepulveda, T. and Viniegra Gonzalez, G. (2006). Production of hydrolytic depolymerizing pectinases. *Food Technology and Biotechnology*, 44: 221-227.

37. Fuller, W. H. and Norman, A. G. (1943). Cellulose decomposition by aerobic mesophilic bacteria from soil. *Journal of Bacteriology*, 46: 273-280.

38. Gade, R. M., Mane, S. S. and Thakur, K. D. (2010). Decomposition of farm wastes by cellulolytic organisms. *Journal of Plant Disease Science*. 5: 154-157.

39. Game, B. C., Deokar, C. D. and More, P. E. (2017). Efficacy of newly developed microbial consortium for composting of rural and urban wastes. *International Journal of Current Microbiology and App Science,* 6: 626-633.

40. Garcia, C., Hernandez, T., Cost, F. and Ayuso, M. (1992). Evaluation of the maturity of municipal waste compost using simple chemical parameters. *Communications in Soil Science and Plant Analysis,* 23: 1501-1512.

41. Gaur, A. C. (1982). Role of mesophilic fungi in composting. *Agricultural Wastes*, 4: 453-460.

42. Ghose, T. K. (1987). Measurement of cellulase activities. *Pure and Applied Chemistry*, 59: 257– 268.

43. Gigliotti, G., Valentini, F., Erriquens, F. G. and Said- Pullicino, D. (2005). Evaluating the efficiency of the composting process; a comparison of different parameters. *Geophysical Research Abstracts*, Vol, 09606, 2nd EGU General Assembly.

44. Girdhari, S. N.and Peshwe, S. A. (2015). Isolation and screening of tannase producing fungi. *International Journal of Current Microbiology and Applied Sciences*, 4: 765-774.

45. Gold, M. H., Glenn, J. and Alic, M. (1988). Use of polymeric dyes in lignin biodegradation assays. *Methods in Enzymology*, 161: 74–78.

46. Golueke, C. G. (1981). Principles of biological resource recovery. *Bio Cycle,* 22: 36-40.

47. Goyal, S. and Sindhu, S. S. (2011). Composting of rice straw using different inocula and analysis of compost quality. *Journal of Microbiology*, 4: 126–138.

48. Guisado, G., Lopez, M. J., M. Garcia, C. V., Suárez-Estrella, F. and Moreno, J. (2012). *Pseudallescheria angusta* a lignolytic microorganism for wood fibres bio modification. *Bio Resources* 7: 464-474.

49. Gupta, S. M., Srivastava, S., Gupta. S. and Ahmed, Z. (2008). Genetic manipulation of fruit ripening: using antisense mRNA strategies. *Journal of Applied Bioscience*, 34: 115–123.

50. Gupta, V. K.,Gaur., Yadava, S. K. and Darmwal, N. S. (2009). Optimization of xylanase production from free and immobilized cells of *Fusarium solani* F7. *Bio Resources*, 4: 932-945.

51. Gutierrez-Correa., Marcel. and Tengerdy, R. P. (1997). Production of cellulase on sugar cane bagasse by fungal mixed culture solid substrate fermentation. *Biotechnology Letters,* 19: 665-667.

52. Haltrich, D., Nidetzky, B. and Kulbe, K. D. (1996). Production of fungal xylanases. *Bioresource Technology,* 15: 137-161.

53. Haltrich, D., Preiss, M. and Steiner, W. (1993). Optimization of a culture medium for increased xylanase production by a wild strain of *Schizophyllum commune*. *Enzyme Microbial Technology*, 15: 137-161.

54. Harazono, K., Yamashita, N., Shinzato, N., Watanabe, Y., Fukastu, T. and Kurane, R. (2003). Isolation and characterization of aromatic degrading microorganisms from the gut of the lower termite *Coptotermes formosanus*. *Bioscience Biotechnology Biochemistry,* 67: 889-892.

55. Haseena, A., Nishad, V. M. and Balasundaran, M. (2016). A consortium of thermophilic microorganisms for aerobic composting. *IOSR Journal of Environmental Science Toxicology and Food Technology,* 10: 2319-2402.

56. Himanen. M. And Hänninen, K. (2011). Composting of bio-waste, aerobic and anaerobic sludges–effect of feedstock on the process and quality of compost. *Bioresource Technology,* 102: 2842-2852.

57. Hue, N.V. and Liu, J. (1995). Predicting Compost Stability. *Compost Science and Utilization,* 3: 8-15

58. Iannotti, D. A., Grebus, M. E., Toth, B. L., Madden, L. V. and Hoitink, H. A. J. (1994). Oxygen respirometry to assess stability and maturity of composted municipal solid waste. *Journal of Environmental Quality*, 23: 1177-83.

59. Iannotti, D. A., Pang, T., Toth, B. L., Elwell, D. L., Keener, H. M. and Hoitink, H. A. J. (1993). A quantitative respirometric method for monitoring compost stability. *Compost science & utilization*, 1: 52-65.

60. Ieshita, P. and Sen, S. K. (2013). Microbial and physico-chemical analysis of composting process of wheat straw. *Indian Journal of Biotechnology*, 12: 120-128.

61. Iglesias, E. and Pérez. V. (1992). Determination of maturity indices for city refuse composts. *Agriculture Ecosystem Environment*, 38: 331-343.

62. Iglesias-Jomenez, E. and Perez-Garcia, V. (1989). Evaluation of city refuse compost maturity. A review, *Biological Wastes*, 27: 115-142.

63. Immanuel, G., Dhanusha, R., Prema, P. and Palavesam, A. (2006). Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. *International Journal of Environmental Science and Technology*, 3: 25-34.

64. Imran, M., Anwar, Z., Irshad, M., Asad, M. J. and Ashfaq, H. (2016). Cellulase production from species of fungi and bacteria from agricultural wastes and its utilization in industry: A Review. *Advances in Enzyme Research*, 4: 44-55.

65. Jahangeer, S., Khan, N., Jahangeer, S., Sohail, M., Shahzad, S., Ahmad, A. and Khan, A. (2005). Screening and characterization of fungal cellulases isolated from the native environmental source. *Pakistan Journal of Botany*, 37: 739- 748.

66. Janani, L., Karthik., Kumar, G. and Bhaskara Rao, K. V. (2011). Screening of pectinase producing microorganisms from agricultural waste dump soil. *Asian Journal of Biochemical and Pharmaceutical Research*, 1: 2231-2560.

67. Kale, R. A. and Zanwar, P. H. (2016). Isolation and screening of cellulolytic fungi. *IOSR Journal of Biotechnology and Biochemistry*, 2: 57-61.

68. Karunakaran, S., Saravanan, A., Dhanasekaran, S., Senbagam, D. and Senthil kumar, B. (2014). Xylanase Production from *Aspergillus niger*. *International Journal of Chem Tech* Research, 6: 4207-4211.

69. Kasana, R. C., Salwan, R., Dhar, H., Dutt, S. and Gulati, A. (2008). A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. *Current Microbiology*, 57: 503-507.

70. Kaur, H. P. and Kaur, G. (2014). Optimization of cultural conditions for pectinase produced by fruit spoilage fungi. *International Journal of Advances in Pharmacy, Biology* and *Chemistry*, 3: 851-859.

71. Kausar, H., Sariah, M., Saud, H. M., Alam, M. Z. and Ismail, M. R. (2010). Development of compatible lignocellulolytic fungal consortium for rapid composting of rice straw. *International Biodeterioration and Biodegradation*, 64: 594–600.

72. Keshab, C., Rintu, B. and Bikas, R. P. (2000). Tannase production by *Bacillus licheniformis*. *Biotechnology Letters*, 22: 767-769.

73. Kheng, P. P. and Omar, I. C. (2005). Xylanase production by a local fungal isolate *A.niger* SMAI solid state fermentation using palm kernel cake (PKC) as a substrate. *Songklanakarian Journal of Science and Technology*, 27: 325-336.

74. Khianngam, S., Pootaeng-on, Y., Techakriengkrai, T. and Tanasupawat, S. (2014). Screening and identification of cellulase producing bacteria isolated from oil palm meal. *Journal of Applied Pharmaceutical Science*, 4: 90-96.

75. Kumar, U., Tapwal, A., Kalkal, P., Varghese, S. and Chandra, S. (2014). Isolation and screening of cellulase producing fungi from forest waste. *International Journal of Pharmaceutical and Biological Archives*, 5: 56-59.

76. Kumar, V. V., Kirupha, S. D., Periyaraman, P. and Sivanesan, S. (2011). Screening and induction of laccase activity in fungal species and its application in dye decolorization. *African Journal of Microbiology Research*, 5: 1261-1267.

77. Kumari Lalitha., Hanuma Sri, M. and Sudhakar, P. (2011). Isolation of cellulose producing fungi from soil, optimization and molecular characterization of the isolate for maximizing the enzyme yield. *World Journal of Science and Technology*, 1: 01-09.

78. Liang, C., Das, K. C. and McClendon, R. W. (2003). The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. *Bioresource technology*, 86: 131-137.

79. Liers, C., Ullrich, R., Steffen, K. Y., Hatakka, A. and Hofrichter, M. (2006). Mineralization of ^{14}C-labelled synthetic lignin and extracellular enzyme activities in wood colonizing ascomycetes *Xylaria hypoxylan* and *Xylaria polymorpha*. *Applied Microbiology and Biotechnology*, 69: 573-579.

80. Lynd, L. R., Weimer, P. J., van Zyl, WH. and Pretorius, I. S. (2002). Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular* Biology *Reviews*, 66: 506-577.

81. Makan, A., Malamis, D., Assobhei, O., Loizidou M. and Mountadar, M. (2012). Multi-criteria decision aid approach for the selection of the best compromise management scheme for the treatment of municipal solid waste in Morocco. *International Journal of Environment and Waste Management*, 7: 96-109.

82. Mandal, A. (2015). Review on microbial xylanases and their applications. *International Journal of Life Sciences*, 4: 178-187.

83. Mandels, M. R., Andreotti, R. and Roche, R. (1976). Measurement of saccharifying cellulose. *Biotechnology Bioenergy Symp*osium, 6: 17-34.

84. Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31: 426-428.

85. Molla, A.H., Fakhrul-Razi, A., Abd-Aziz, S., Hanafi, M. M., Alam, M.Z. (2001). *In vitro* compatibility evaluation of fungal mixed culture for bioconversion of domestic wastewater sludge. *World Journal of Microbiology and Biotechnology*, 17: 849-856.

86. Mukhlis, H., Mohd Saud., Sariah, M., Razi Ismail, M., Habib, S. H. and Kausar, H. (2013). Potential lignocellulolytic *Trichoderma* for bioconversion of oil palm empty fruit branches. *Australian Journal of Crop Science*, 7: 425-431.

87. Murugan, K., Saravanababu, S. and Arunachalam, M. (2007). Screening of tannin acyl hydrolase producing tannery effluent fungal isolates using simple agar plate and SmF process. *Bioresource Technology*, 98: 946-949.

88. Nair, S. G., Sindhu, R. and Shashidhar, S. (2008). Fungal xylanase production under solid state and submerged fermentation conditions. *African Journal of Microbiology Research*, 2: 82-86.

89. Nakasaki, K. and Marui, T. (2011). Progress of organic matter degradation and maturity of compost produced in a large-scale composting facility. *Waste Management and Research*, 29: 574–581.

90. Nakumura, K. and Kappamura, K. (1982). Isolation and identification of crystalline cellulose hydrolyzing bacterium and its enzymatic properties. *Journal of Fermentation Technology*, 60: 343-348.

91. Nelson, D.W. and L.E. Sommers. (1996). Total carbon, organic carbon, and organic matter. In: Methods of Soil Analysis, Part 2, 2nd ed., A.L. Page *et al*., Ed. Agronomy. 9:961-1010. Am. Soc. of Agron., Inc. Madison, WI.

92. Nishida, T., Yoshinori, K., Mimura, A. and Takahara, Y. (1988). Lignin biodegradation by wood-rotting fungi I. Screening of lignin degrading fungi. *Mokuzai Gakkaishi*, 34: 530–536.

93. Oyeleke, S. B., Oyewole, O. A., Egwim, E. C., Dauda, B. E. N. and Ibeh, E. N. (2012). Cellulase and pectinase production potentials of *Aspergillus niger* isolated from corn cob. *Bayero Journal of Pure and Applied Sciences*, 5: 78-83.

94. Pandey, A., Soccol, C. R. and Mitchell, D. (2000). New development in solid state fermentation. *Process Biochemistry*, 35: 1153-1169.

95. Paradelo, R., Moldes, A. B. and Barral, M. T. (2013). Evolution of organic matter during the mesophilic composting of lignocellulosic winery wastes. *Journal of environmental management*, 116: 18-26.

96. Paranthaman, R., Vidyalakshmi, J., Indumathi, J. and Singaravadevi, K. (2009). Biosynthesis of tannase and simultaneous determination of phenolic compounds in *Aspergillus niger* fermented paddy straw by HPLC. *Global Journal of Biotechnology and Biochemistry*, 4: 93-97.

97. Pascual, J. A., Garcia, C., Hernandez, T. and Ayuso, M. (1997). Changes in the microbial activity of an arid soil amended with urban organic wastes. *Biology and Fertility of Soils*, 24: 429–434.

98. Perez, J., Munoz-Dorado, J., de la Rubia, T. and Martinez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose, and lignin: an overview. *International Microbiology*, 5: 53-63.

99. Pointing, S. B. (1999). Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. *Fungal Diversity*, 2: 17-33.

100. Pointing, S. B., Parungao, M. M. and Hyde, K. D. (2003). Production of wood-decay enzymes, mass loss, and lignin solubilization in wood by tropical Xylariaceae. *Mycological Research*, 107: 231–235.

101. Polizeli, M. L. T. M., Rizzatti, A. C. S., Monti, R., Terezni, H. F., Jorge, J. A. and Amorim, D.S. (2005). Xylanases from fungi: properties and industrial applications. *Applied Microbiology and Biotechnology*, 67: 577-591.

102. Polyanna, H., Lima, D., Fernandes, M., Neves, R., Souza-Motta, C. and Porto, A. (2011). Isolation of cellulolytic fungi from waste of castor (*Ricinus Communis*). *Current Microbiology*, 62: 1416-1422.

103. Priya, V. and Sashi, V. (2014). Pectinase enzyme producing microorganisms. *International Journal of Scientific and Research Publications*, 4: 1-4.

104. Rahna, K. R., Preethi, N. and Balasaravanan, T. (2012). Isolation, identification and characterization of efficient cellulolytic fungi from natural resources. *International Journal of Microbial Resource Technology*, 1: 379-387.

105. Raj, D. and Antil, R. S. (2011). Evaluation of maturity and stability parameters of composts prepared from agro-industrial wastes. *Bioresource Technology*, 102: 2868-2873.

106. Rajasekhar, L., Sain, S. K. and Divya, J. (2016). Evaluation of microbial consortium for plant health management of chick pea. *International Journal of Applied Biology and Pharmaceutical Technology*, 7: 115-121.

107. Ranalli, G., Bottura, G., Taddei, P., Garavani, M., Marchetti, R. and Sorlini, C. (2001). Composting of solid and sludge residues from agricultural and food industries- bio indicators of monitoring and compost maturity. *Journal of Environmental Science and Health*, Part A. 36: 415-436.

108. Reddy, L. P. and Sreeramalu, A. (2012). Isolation, identification and screening of pectinolytic fungi from different soil samples of Chittoor district. *International Journal of Life Sciences Biotechnology and Pharma Research*, 1: 185-213.

109. Saharinen, M. H., Vuorinen, A. H. and Kostikka, M. (1996). Effective cation exchange capacity of manure-straw compost of varying stages determined by the saturation-displacement method. *Communications in Soil Science and Plant Analysis*, 27: 2917-23.

110. Said-Pullicino, D., Erriquens, F. G. and Gigliotti, G. (2007). Changes in the chemical characteristics of water-extractable organic matter during composting and their influence on compost stability and maturity. *Bioresource Technology*, 98: 1822–1831.

111. Sanchez, C. (2009). Lignocellulosic residues: biodegradation and bioconversion by fungi. *Biotechnology Advances*, 27: 185-194.

112. Sanchez-Monedero, M. A., Roig, A., Cegarra, J. and Bernal, M. P. (1999). Relationships between water-soluble carbohydrate and phenol fractions and the humification indices of different organic wastes during composting. *Bioresource Technology*, 78: 301–308.

113. Sanchez-Monedero, M. A., Roig, A., Martinez-Pardo, C., Cegarra, J. and Paredes, C. (1996). A microanalysis method for determining total organic carbon in extracts of humic substances. Relationships between total organic carbon and oxidable carbon. *Bioresource Technology*, 57: 291-295.

114. Sandhya, R. and Kurup, G. (2013). Screening and isolation of pectinase from fruit and vegetable wastes and the use of orange waste as a substrate for pectinase production. *International Research Journal of Biological Sciences*, 2: 34-39.

115. Sanghi, A., Garg, N., Gupta, V. K., Mittal, A. and Kuhad, R. C. (2010). One-step purification and characterization of cellulase-free xylanase produced by alkalophilic *Bacillus subtilis*. *Brazilian Journal of Microbiology*, 41: 467-476.

116. Sapek, B. and Sapek, A. (1999). Determination of optical properties in weakly humified samples. In: Dziadowiec, H., Gonet, S.S. (Eds.), The Study of Soil Organic Matter—the Methodical Guide. Warszawa, Poland (in polish).

117. Sarkar, P., Meghvanshi, M. and Singh, R. (2011). Microbial consortium: A new approach in effective degradation of organic kitchen wastes. *International Journal of Environmental Science and Development*, 2: 67-71.

118. Sarker, T., Mannan, M. A., Mondal, P. C., Kabir, A. H., Parvez, S. M. and Alam, M.F. (2013). Physico-chemical profile and microbial diversity during bioconversion of sugarcane press mud using bacterial suspension. *Notulae Scientia Biologicae*, 5: 346-353.

119. Sasikumar, V., Priya, V., Shiv Shankar, C. and Sathish Sekar, D. (2014). Isolation and preliminary screening of lignin degrading microbes. *Journal of Academia and Industrial Research*, 3: 291-294.

120. Saxena, R. K., Sharmila, P. and Singh, V. P. (1995). Microbial degradation of tannins. *Progress in Industrial Microbiology,* 32: 259-270.

121. Selim, S. M., Zayed, M. S. and Atta, H. M (2012). Evaluation of phytotoxicity of compost during composting process. *Nature and Science,* 10: 69-77.

122. Sharma, N., Buragohain, P., Tandon, D. and Kaushal, R. (2013) Comparative study of potential cellulolytic and xylanolytic bacteria isolated from compost and their optimization for industrial use. *Journal of Agroalimentary Processes and Technologies,* 19: 284-297.

123. Silva, I. S., Cristiano, R. M., Elisangela, F., Eder, C. S. and Lucia, R. D. (2010). Degradation of lignosulfonic and tannic acid by lignolytic soil fungi cultivated under micro aerobic conditions. *Brazilian Archives of Biology and Technology,* 53: 693-699.

124. Singh, N. and Abraham, J. (2013). Isolation of laccase producing fungus from compost soil and partial characterization of laccase. *Advances in Applied Science Research*, 4: 91-98.

125. Sjöström, E. (1993). Wood Chemistry. Fundamentals and Applications, 2nd edition, Academic Press, San Diego, CA, USA, 293 p.

126. Smith, D.C. and Hughes, J. C. (2001). A simple test to determine cellulolytic activity as indicator of compost maturity. *Communications in Soil Science and Plant Analysis,* 32: 1375-749.

127. Soares, M. M. C.N., Silva, R.D. and Gomes, E. (1999). Screening of bacterial strains for pectinolytic activity; characterization of the poly galactouronase produced by *Bacillus* sp. *Revista de Microbiologica*, 30: 229-303.

128. Srivastava, A. and Kar, R. (2009). Characterization and application of tannase produced by *Aspergillus niger* on pomegranate rind. *Brazilian Journal of Microbiology,* 40: 782-789.

129. Stratton, M. L. Barker, A.V. and Rechcigl, J. E. (1995) Compost. In J.E. Rechcigl (ed.) Soil Amendments and Environmental Quality. CRC Lewis Publ., New York. p. 250–309.

130. Stratton, M. L. and Rechigl, J. E. (1997). Compost application to rye grass. Agron, Abst,. SII -031-P, Anaheim, CA.

131. Sundberg, C., Yu, D., Whittle, I. F., Kauppi, S., Sven Smårs, S., Insam, H., Romantschuk, M. and Jönsson, H. (2013). Effects of pH and microbial composition on odour in food waste composting. *Waste Management,* 33: 204-211.

132. Sushir, M. L., Narkhede, K. P., Salunkhe, R. C. and Patil, S. R. (2016). Isolation and screening of xylanolytic fungi from natural habitat in Khandesh region of Maharashtra, India. *Journal of Chemical and Pharmaceutical Research*, 8: 581-586

133. Taneja, K., Saurabh, G. and Kuhad, R. C. (2002). Properties and application of a partially purified alkaline xylanase from an alkalophilic fungus *Aspergillus nidulans* KK99. *Bioresource Technology*, 85: 39-42.

134. Teather, R. M. and Wood, P. J. (1982). Use of congo red polysaccharide interactions complex formation between congo red and polysaccharide in detection and assay of polysaccharide hydrolases. *Methods in Enzymology*, 160: 59-74.

135. Tiquia, S. M., Tam, N. F. Y., and Hodgkiss, I. J. (1996). Microbial activities during composting of spent pig-manure sawdust litter at different moisture contents. *Bioresource Technology*, 55: 201-206.

136. Varghese, L. K., Rizvi, A. F. and Gupta, A. K. (2013). Isolation, screening and biochemical characterization of pectinolytic microorganisms from soil sample of Raipur city. *Journal of Biological and Chemical Research*, 30: 636-643.

137. Walkley, A. and Black, I. A. (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science*, 37: 29-37.

138. Wanga, P., Changaa, C. M., Watsonb, M. E., Dickb, W. A., Chenc, Y. and Hoitinka, H. A. J. (2004). Maturity indices for composted dairy and pig manures. *Soil Biology and Biochemistry*, 36: 767-776.

139. White, T. J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications. (Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. eds). Academic Press, New York, USA: 315–322

140. Wu, L., Ma, L. Q. and Martinez, G. A. (2000). Comparison of methods for evaluating stability and maturity of biosolids compost. *Journal Environmental Quality*, 29: 424–429.

141. Zmora-Nahum, S., Markovitech, O., Tarchitzky, J. and Chen, Y. (2005). Dissolved organic carbon (DOC) as a parameter of compost quality. *Soil Biology and Biochemistry*, 371: 2109-2116.

142. Zucconi, F. and de Bertoldi, M. (1987). Compost specification for the production and characterization of compost from municipal solid waste. In: de Bertoldi, M., Ferranti, M.P., Hermite, P.L., Zucconi, F. (Eds.), Compost: Production, Quality and Use. Elsevier Applied Science Publishers, pp: 30–50.

143. Zucconi, F., Monaco, A., Forte, M. and De Bertoldi, M. (1985). Phytotoxins during the stabilization of organic matter. In Composting of Agricultural and other Wastes, ed.. Gasser, J. K. R. Elsevier Applied Science Publishers, London & New York: 73-85.

Appendix

APPENDIX

Iodine Solution

Iodine	1.0g
Potassium iodide	2.0g
Distilled water	300ml

Dintrosalicylic acid (DNS) Reagent:

Solution- A:	Sodium Hydroxide	10g
Solution -B:	Dintrosalicylic acid	10g
	Phenol crystals	2.0g
	Sodium Potassium Tartarate	0.2g

Dissolved solution-B in solution-A

Solution-C:	Sodium sulphite	5.0g
	Distilled Water	100ml
Working Reagent:	Solution-B:	99ml
	Solution-C:	1ml

Media Composition

Xylan Basal Medium (XBM) (g l^{-1} in distilled water):

Ammonium tartrate	5
Yeast Extract	0.1
KH_2P0_4	1
$CaCl_2.2H_20$	0.001
$MgS0_4.7H_20$	0.5

Lignin Basal Medium (LBM) (g t1 in distilled water):

KH_2P0_4	1
Yeast Extract	0.01
$C_4H_{I2}N_20_6$	0.5
$CuSO_4.5H_20$	0.001
$MgS0_4 \cdot 7H_20$	0.5
$Fe_2(S0_4)_3$	0.001
$CaCl_2 \cdot 2H_20$	0.01
$MnSO_4.H_20$	0.001

Nutrient Agar:

Beef extract	10.0g
NaCl	5.0g
Peptone	10.0g
Distilled water	1.0L
Agar	20.0g
pH	7.0-7.5

Potato Dextrose Agar (PDA):

Potato	200g
Dextrose	20g
Agar	20g
Distilled water	1000ml
pH	5.6

Sabouraud's Agar:

Peptone	10g
Dextrose	40g

Agar	15g
Distilled water	1000ml
pH	5.6

Czapek DoxAgar (CDA) :

$NaNO_3$	2.0g
KH_2PO_4	1.0g
$MgSO_4.7H_2O$	0.5g
KCl	0.5g
$FeSO_4.7H_2O$	0.1g
Sucrose	30.0g
$ZnSO_4.7H_2O$	10mg
$CuSO_4$	10mg
Agar	20g
Distilled water	1000ml
pH	7.0

Mandel and Reese Agar :

Peptone	1.0g
KH_2PO_4	2.0g
Urea	0.3g
$(NH_4)_2SO_4$	1.4g
$CaCl_2$	0.3g
$MgSO_4.7H_2O$	0.3g
$FeSO_4$	0.005g
$MnSO_4$	0.0016g
ZnCl	0.0017g

Carboxy Methyl Cellulose	10g
Yeast extract	0.25g
Agar	20
Distilled water	1000ml
pH	5.3

Carboxy Methyl Cellulose (CMC) Agar:

Carboxy methyl cellulose	5.0g
K_2HPO_4	1.0g
$MgSO_4$	0.2g
$CaCl_2$	2-5mg
$Fe_2(SO_4)_3$	2mg
Agar	15-20g
Distilled water	1000ml
pH	7-7.5

Tannic Acid Malt Extract Agar (TAMEA):

Malt extract	15g
Tannic acid	10g
Agar	30g
pH	6-6.5