# EFFICACY OF LIGNO-PHENOLIC COMPOST IN THE SUPPRESSION OF SOIL BORNE PLANT PATHOGENS

By

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(2010-21 -108)


## THESIS

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## DECLARATION

I hereby declare that this thesis entitled **"EFFICACY OF LIGNO-PHENOLIC COMPOST IN THE SUPPRESSION OF SOIL BORNE PLANT PATHOGENS"** is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title of any other University or Society.

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Certified that this thesis, entitled " **EFFICACY OF LIGNO-PHENOLIC COMPOST IN THE SUPPRESSION OF SOIL BORNE PLANT PATHOGENS**" is a bonafide record of research work done independently by **Mrs. Gleena Mary C.F.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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# *Introduction*

# 1. INTRODUCTION

Solid waste management is one of the biggest environmental challenges facing the world today. Lignocellulosic biomass from plants is the most abundant group of agricultural residue in the world which accounts for more than 60 per cent of the total biomass (Kuhad *et al.*, 1997). Accumulation of these lignocellulosic wastes has gained momentum due to rapid industrialisation and economic development by the utilization of plant based products in various industries.

Kerala, God's own country with plethora of naturally available species of medicinal plants encouraged by climate, environment, topography and culture is well known for traditional ayurvedic medicine from times immemorial. Modernisation of ayurvedic practice and promotion of Kerala tourism based on ayurvedic wellness therapy has revolutionized ayurvedic pharmaceutical industry with increased demand and production of ayurvedic medicines. This demand and production of ayurvedic medicines has resulted in the generation of large quantum of agrowastes and left over materials after the extraction of valuable alkaloids and their derivates which include woody stumps and roots of medicinal plants. Some of the medicinal plants are reported to have inhibitory effect on plant pathogens, due to the presence of phenols and alkaloids, but make the composting process difficult and laborious.

Coconut is the major oil seed and cash crop of Kerala. Coir pith, also known as coir dust, the major by product of coir fibre extraction is abundant in lignin (20-40%), cellulose (40-50%), hemicellulose (15-35%) and (2- 4%), protein (Sjostrom, 1993). Coir pith production in India is estimated to be 2.5 million tons and of this 60 per cent is from Kerala. Decomposition of coir pith is rather slow due to its high C: N ratio of 75 -186:1 and exceptionally high content of lignin. Likewise, an estimated production of 8 lakh tons of cashew apple is discarded every year and leaf litters are accumulating to the tune of 4-5 t/ ha /year (Mini *et al.*, 2005). The practice of disposal of solid wastes by burning and land

filling are causing high environmental problems and the negative effects of these existing wastes can be converted to beneficial biofertilizers.

Composting is the most economical and sustainable option for the management of such organic wastes which involves microbiological process which goes on naturally with the help of native microorganisms in the soil. Composting of plant residues depend upon the chemical constituents of substrates and lignin –tannin rich ayurvedic waste, coconut by products, leaf litters of cashew, mango and teak take longer time for degradation. Delay in degradation and disposal results in accumulation of these agrowastes. Therefore, the strategy of the composting process has to be manipulated for rapid decomposition of these organic materials which can be attained by addition of selected lignin, tannin and cellulose degraders. The present study is under taken to identify efficient native microbial degraders of these components to develop a consortium to accelerate rapid decomposition of the various ligno-phenolic agro wastes and to improve the quality of compost products by increasing nutrient contents which enhance plant growth and disease resistance.

Now a days, greater thrust has been given for microbial consortium, since it consists of microbes with different biochemical and physiological capabilities which provides better degradation by their synergistic effect and multiple mode of action. Thus, the use of microbial consortium helps to reduce the composting period and enable the potential conversion of waste into value added products in shorter time and thus the turnover rate can be increased. Moreover, these microbial degraders may also help in reducing harmful biochemical contents in ligno-phenolic agrowastes, thereby making the substrates suitable for improving the soil fertility.

Bacterial wilt caused by *Ralstonia solanacearum* is the major constraint in the production of tomato and many other crops in tropics, subtropics and warm temperate regions of the world (Buddenhagen and Kelman, 1964; Hayward, 1991). The pathogen has a wide host range of more than 200 species in 50

families (Hayward, 1994). The disease management is being attempted with chemicals, inter cropping, crop rotations and by the use of resistant cultivars. The genetic diversity of the pathogen often overcomes the crop resistance (Wang *et al.* 1998) and furthermore, the use of chemicals has its adverse effects both on environment as well as on beneficial microorganisms.

Amaranthus is one of the most important leafy vegetable which enriches our diet with its exceptionally high fibre and mineral contents. The crop is well suited to Kerala condition, as it can be grown as intercrop in coconut gardens, fits well with other crops in rotations and respond well to organic and inorganic fertilisers. The cultivation of the crop is hampered during monsoons by the heavy infestation of leaf blight caused by *Rhizoctonia solani* which results in considerable economic loss owing to reduction in the marketability of the produce. Being a short duration leafy vegetable, chemical control measures are not advisable for the management of this disease. Hence the use of organic products has got significant importance in the management of this disease.

At this context, the search for alternatives to control the soilborne pathogens with high efficiency, low cost and limited environmental impact has been arisen for maintaining sustainable agricultural practices. Keeping view of these aspects, the present study was undertaken with the following objectives.

1. To develop a consortium of microbial degraders for rapid composting of ligno-phenolic agrowastes.
2. To study the effect of these ligno-phenolic compost products in the suppression of soil borne pathogens.

# Review of literature

# 2. REVIEW OF LITERATURE

With global population expected to climb into tens of billions within the next century, the rapid increase in the volume of waste and the issue of waste disposal has become even more crucial in recent times (Wouters *et al.*, 2005). Solid wastes generated from various sources include biodegradable matter (30-55 %), inert non biodegradable matter (40-45%) and recyclable matter (5-10%). Tandon (1995) reported that, total annual waste biomass of India was about 2500 million tons, which include municipal solid wastes, agricultural residues, cattle and poultry manures and agro industrial wastes. Thimmaiah and Bhatnagar (1999) reported that, India generated 345 million tons of solid waste annually, of which approximately 25million tons were municipal solid wastes and 320 million tons were agricultural residues. According to Seth (2010), India generated 38 million tons of municipal solid wastes annually. Hamza (1989) observed that uncontrolled discharge and open dumping of agro industrial wastes contributed to appreciable environmental deterioration which was manifested as serious hazards to public health, pollution of air and water bodies, increased eutrophication due to excessive discharge of nutrients, depletion of dissolved oxygen in water surface, offensive odors due to anaerobic decomposition of organic residues and unhealthy conditions in waste storage and land disposal sites.

## 2.1. Lignocellulosic agrowastes

Lignocellulosic biomass from plants are the most abundant agricultural residues in the world and it accounts for more than 60 per cent of the total biomass production (Kuhad *et al.*, 1997).

Agricultural and industrial wastes including ayurvedic waste, coir pith, rice bran, rice husk, rice straw, sugarcane trash, bagasse, press mud etc. have a huge potential for recycling nutrient elements. Animal wastes such as cattle dung, elephant dung, poultry waste, rural and urban organic wastes can also be used for bioconversion as organic manure.

Indian ayurvedic medicinal plant based industry is growing at the rate of 7-15 per cent annually. In Kerala, on an average, annual consumption of raw drugs is more than 400 tons and among the 14 districts raw drug consumption is found to be highest in Thrissur and Malappuram due to the presence of large manufacturing units (Sasidharan and Muraleedharan, 2009). An ayurvedic pharmaceutical unit in Thrissur produces biodegradable wastes of more than 400 formulations and about one tonne of waste is being generated per day for want of disposal.

Coir pith is a highly lignocellulosic material obtained as byproduct of coconut coir industry and can be effectively used as organic manure by composting or such bioconversions. In India, 0.7 million tons of coir pith is accumulated annually for want of disposal (Thampan, 1987). Nagarajan *et al.* (1985) also reported the accumulation of one ton coir pith for every 1000 husks used in coir industry. It is estimated that, at present there is an accumulated stock if 10 million tons of coir pith in southern states of India (Ghosh *et al.* 2007). Mallinga *et al.* (1996) observed that, in spite of their limited use as soil conditioners, the coir dust produced is so enormous making its disposal difficult due to its lignocellulosic nature and slow degradation in natural environment.

Likewise, cashew processing industry discards around 8 lakh tons of cashew apple waste every year and cashew leaf litters gets accumulated to the tune of 4-5t/ha/year for want of disposal (Mini *et al.*, 2005).

**2.2. Composting of agro wastes**

Composting is the process of conversion of organic substances by a mixed population of microorganisms such as fungi, bacteria and actinomycetes which perform their specific roles in increasing macro and micronutrients resulting in the loss of organic carbon and resulting in volume reduction.

During composting, organic matter from the biodegradable wastes is microbiologically degraded, resulting in a final product containing stabilized carbon, nitrogen and other nutrients in the organic factors. Anbuselvi and Rebecca

(2009) observed that, the organic wastes generated from the industrial and agricultural sectors can be converted into beneficial products such as biofertilizer and as soil conditioner through the process of biocomposting.

### 2.3. Factors influencing composting process

Temperature, moisture content, oxygen content, material particle size and nature of feed stock, particularly C:N ratio are the key factors need to be controlled for composting to occur in an optimum manner (Pace *et al.*, 1995; Evans, 2001; Last, 2006).

#### 2.3.1. Temperature

Several workers demonstrated that, fermentations operating at thermophilic temperatures resulted in more rapid degradation of organic matter (Hashimoto *et al.*, 1981; Kimchie *et al.*, 1988).

Gowda (1996) reported that, during decomposition of wastes, the microbes consume more $O_2$ to break down the organic compound and release heat energy through respiration process, which caused the rise in temperature during decomposition of organic matter. At high temperature (40-70°C), all thermophilic microbes, which are more efficient in degradation will multiply and decompose the material faster.

Gaur (1996) also observed a great deal of exothermic energy being released during the process, generating substantial amounts of heat, thus raising the temperature in heaps and creating favorable conditions for thermophilic micro organisms. He also noted that, if the temperature exceeded 65°C to 75°C, the microbial activity ceased due to thermal killing of organisms.

According to Nair (1997) peak value of temperature, 66.3°C and pH 7.4 were attained during the thermophilic stage of composting. Eghball *et al.* (1997) reported a temperature of 65°C within 24 h at all depths of the compost pile during the composting of cattle feed manure. The elevated temperature of 40-50°C found during the thermophilic stage is essential for rapid degradation of

lignocelluloses, as the thermophilic fungi and actinomycetes involved in this process thrive at high temperature (Tuomela *et al.*, 2000).

Crowe *et al.* (2002) noted that, temperature of the biomass increased due to the microbial activity and the insulation properties of the piled material. The temperature often reaches 65°C-75°C within few days (thermophilic phase) and then decline slowly. He also observed that, this high temperature furthers the elimination of pathogens and weed seeds. Peigne and Girardin (2004) also reported the release of heat by the oxidative action of microbes during the conversion of organic matter.

Preetha (2003) recorded high temperature values on $10^{th}$ day of composting of ayurvedic waste with *Schizophyllum commune and Pleurotus platypus* and the peak temperature later reduced at compost maturity. Pandey (1997) suggested that, temperature maintained between 60°C and 70°C for 24 h was necessary to kill the pathogens and weed seeds during composting process. Maximum activity of cellulolytic enzymes was observed at a temperature of 40°C (Gautam *et al.*, 2010).

### 2.3.2. Moisture

According to Krishnamurthy (1978) moisture content of the composting material has an important role in satisfactory decomposition. A structure allowing oxygen diffusion through the pile and moisture content suitable to support the metabolic processes of the microorganisms is necessary for composting (Pace *et al.*, 1995; Last, 2006).

Gaur (1986) reported that, moisture content of 50-60 per cent was favourable for composting and this moisture percentage enhances the biodegradation of the substrate. Rao *et al.* (1995) observed moisture content of 70 per cent was favorable for more effective composting as indicated by higher level of mineralization of poplar wood. Optimum moisture content of 60 per cent for effective composting of organic wastes with microbial consortium was reported

by various researchers (Pointcelot, 1974; Goyal and Sindhu, 2011; Mohan and Ponnusamy, 2011).

### 2.3.3. Aeration

Jakobsen (1995) pointed out that, good aeration during decomposition promote conversion of carbohydrates into carbon dioxide and water. Incomplete aeration may result in accumulation of acetic acid and is not suitable for plant growth.

Numerous studies showed that, at adequate aeration, the temperature in the compost pile increased to higher than 60°C. This accelerated the decomposition process and resulted in complete destruction of pathogens, parasites and weed seeds (Bishop and Godfrey, 1983; Parr *et al.*, 1994)

Gowda (1996) reported that, in aerobic decomposition, the organic matter has undergone complete degradation, mineralization and formed non toxic components *viz.* $CO_2$, water, heat (energy) and water soluble mineral forms of nutrients and humus (ligno-proteins), whereas, in anaerobic decomposition, due to in sufficient oxygen supply, only few anaerobic bacteria decomposed and formed intermediary organic compounds with foul smell and phytotoxicity.

Yu *et al.* (2007) evaluated two composting mixtures by the addition of grape stalks as bulking agent to one of the pile to study their effect on the composting process and final compost quality. The particular compost pile with grape stalks showed faster composting and the compost product had superior chemical and physical properties as compared to the other.

## 2.4. Microbial degradation of lignocellulosic agrowastes

Microorganisms in their natural habitat are crucial to the functioning of the world's ecosystems and the major contributors to biochemical cycles. Beneficial micro organisms in the decomposition of organic matter, in remediation of pollutants and in increased nutrient availability leads to improved soil fertility and

crop productivity (Dileep and Dixit, 2005; Weyens *et al.*, 2009; Sindhu *et al.*, 2011).

Gaur *et al.* (1982) observed one month reduction in the composting period of mixed substrates consisting of jowar stalk, and wheat (5:3) and jamun leaves inoculated individually with mesophilic cellullolytic fungi, *Aspergillus niger, Aspergillus* sp., *Trichoderma viride* and *Penicillium* sp.

Kerem *et al.* (1992) studied solid state fermentation of cotton stalks by two white rot fungi, *Pleurotus ostreatus and Phanerochaete chrysosporium* and they observed 55 per cent degradation of cotton stalks with *P. chrysosporium* within 15 days, whereas, *P. ostreatus* showed only 20 per cent degradation of organic matter by 20 days. Makkar *et al.* (1994) observed biodegradation of tannin in oak leaves by *Sporotrichum pulverulentum* .

Savithri and Khan (1994) found *Trichoderma* and *Aspergillus* spp. as potent degraders of coir pith. Preetha (2003) carried out precomposting of ayurvedic waste with *P. platypus* and *S. commune* and observed that, both were equally effective in reducing the precomposting period. Rajan *et al.* (2005) studied the biosoftening of coir fibre by treatment with different microorganisms *viz. Pseudomonas putida, P. chrysosporium, Aspergillus flaviceps and Trametes hirsuta.* Among these, *P. putida* and *P. chrysosporium* showed maximum preferential degradation of lignin, corresponding to high manganese peroxidase activity.

Sushama (2005) observed aerobic composting of coir pith with cow dung, goat manure, *Pleurotus* sp. along with water hyacinth, within a period of 110 days. Vermicomposting of cashew apple residue and leaf litters was carried out by Mini *et al.* (2005) and reported that, apple residues and leaf litters of cashew could be effectively utilized for the production of vermicompost and was a good ameliorant for the acid soils of Kerala.

## 2.5. Role of enzymes in degradation of agrowastes

Treatment of agricultural residues with either lignocellulolytic enzymes or microorganisms could lead to more efficient degradation of these waste materials, promote recycling of nutrients in the environment and reduce the impact of waste accumulation (de Vries and Visser, 2001).

Mc Carthy and Broda (1984) screened large number of actinomycetes for their ability to degrade ball milled straw or to grow on lignin related phenolic compounds and found that, actinomycetes belonging to *Thermomonospora* and *Micromonospora* were effective in degrading these materials. They also noticed that, non-straw degrading strains, *T. mesophila* and *Streptomyces* sp. utilized phenolic compounds produced from lignocellulosic substrates.

Thormann *et al.* (2002) studied *in vitro* decomposition of cellulose, starch and tannic acid by nine species of fungi isolated from *Sphagnum fuscum.* Of these only *Oidiodendron maius* and *O. scytaloides* recorded positive reaction for polyphenol oxidase and degradation of tannic acid. They also observed a decomposition of 10.2 per cent in spruce wood chips by *Bjerkandrea adusta* and 5.1per cent in *Sphagnum fuscum* by *Sordaria fumicola* after eight weeks.

Sudto *et al.* (2008) examined *Bacillus subtilis*, *Escherichia coli* and *Rhizobium* sp. for carboxy methyl cellulase production and found that, all strains showed growth in the medium containing carboxy methyl cellulose as sole carbon source but no difference was noticed in the enzyme activities of the tested bacteria. They also noticed high bacterial population in the media containing pineapple peel, vegetable residue and corn cob as carbon sources and corn cob stimulated maximum production of the enzyme.

Nurkanto (2010) isolated and characterized seven actinomycetes from soil and studied the growth and cellulase activities of these organisms at different pH. *Streptomyces* and *Actinoplanes* showed significant correlation for cellulase activities, cell growth and pH and the highest enzyme activities were noticed in *S. bobili* at 96 h of incubation.

*In-vitro* enzymatic assay for cellulose degradation by *Aspergillus* spp. was conducted by Ghanbary *et al.* (2010). The results showed that, all *Aspergillus* isolates had the ability to degrade cellulose and there was no significant difference in cellulose activity between the isolates belonging to one species. However, significant variations were found between *Aspergillus* spp. Among the tested species, *A. niger* and *A. niveus* showed highest potential of cellulose degradation.

Wahyudi *et al.* (2010) isolated bacteria from buffalo and horse gastro-intestinal tract and elephant dung having potential to degrade lignin, xylan and cellulose. Maximum lignolytic activity was observed in the bacteria from buffalo caecum, xylanolytic from horse's caecum and cellulolytic from buffalo colon and these superior isolates were identified as *Enterococcus casseliflavus* or *E. gallinarum.*

Bandounas *et al.* (2011) studied lignolytic potential of soil bacteria, *Pandoraea norimbergensis, Pseudomonas* sp. and *Bacillus* sp. by growth on high and low molecular weight lignin fractions, utilization of lignin associated aromatic monomers and degradation of lignolytic indicator dyes. Among the three, *P. norimbergensis* and *Pseudomonas* sp. exhibited growth on lignin fractions with limited dye decolorizing capacity whereas, *Bacillus* sp. showed least efficient growth on lignin fractions but extensive dye decolorizing capacity with preference to recalcitrant phenothiazine dyes (Azure- B, Methylene Blue and Toluidene- Blue O). Raghuwanshi *et al.* (2011) isolated a strain of *Bacillus sphaericus* which was found to produce high amount of tannase enzyme under unoptimised conditions.

Decomposition of coir pith waste by fresh water cyanobacterium, *Oscillatoria annae,* was reported by Anandraj *et al.* (2012). The degradation was confirmed by the presence of lignolytic enzyme, polyphenol oxidase and by the quantification of biochemical parameters such as reducing sugars, phenols, proteins, lipids, nucleic acids, nitrate and ammonia in the samples.

Eight isolates of cellulose degrading bacteria (CDB) were isolated from four different invertebrates (termite, snail, caterpillar and bookworm) and tested for cellulose degradation. Two isolates, CDB-8 and 10 exhibited maximum clear zones on cellulose congo red agar media and further enzymatic assays also confirmed their capacity to degrade filter paper cellulose with maximum degradation of 65.7 per cent for CDB-8 (Gupta *et al.*, 2012).

Marine microorganisms, *Bacillus pumilus* and *Mesorhizobium* sp. tested on 18 different agrowastes having low starch and carbohydrates and high amount of lignocellulosic complex, showed maximum degradation in rice straw, ragi straw, paper and eucalyptus. Maximum cellulose degradation was observed in 5$^{th}$ week whereas, lignin degradation was maximum in 3$^{rd}$ week. Among the two, *B. pumilus* exhibited high enzymatic hydrolysis of the substrates compared to *Mesorhizobium* sp. (Prasad *et al.*, 2014).

**2.6. Biochemical changes during composting**

Substrates with primary components of plant materials such as cellulose, hemicelluloses and lignin are rather difficult to degrade and reduce the availability of other polymers by means of physical restriction (Ladisch *et al.*, 1983).

Several studies have showed the importance of microbial population especially the lignocellulose degrading microorganisms in the compost which helped in enhancing lignocellulose waste decomposition (Beguin and Aubert, 1994; Hart *et al.*, 2002; Lu *et al.*, 2004).

Bhat *et al.* (1997) studied tannin degradation by the fungi, *Aspergillus niger* Van Tiegham, which indicated that, the fungus was able to tolerate high levels of tannins and utilized it as sole carbon source upto 15per cent in the media without growth inhibition. Singh and Sharma (2002) reported significant decrease in cellulose, hemicelluloses and lignin contents of wheat straw during pre decomposition with a consortium of microbes (*Pleurotus sajorcaju, T. harzianum, Azotobacter chroococcum* and *A. niger*) in 40 days. The per cent of

cellulose, hemicellulose and lignin in predecomposed wheat straw were 27.88, 16.79 and 10.48 respectively.

Preetha (2003) noticed reduction in cellulose and lignin contents of ayurvedic waste from 35.7 to 18.8 and 37.9 to 28.2 per cent respectively in the vermicompost, pretreated with biotic agents. Cruz-Hernandez *et al.* (2005) noted 67.70 and 70 per cent degradation of hydrolysable tannins (tannic acid) with *A. niger* and *Penicillium commune* and the fungi recorded 79.33 and 76.35 per cent degradation of condensed tannin, catechin respectively.

Lu *et al.* (2005) observed cellulose degradation by four groups of mixed cultures of mesophilic bacteria. The bacterial groups initiated cellulose degradation in 3 days and degraded 23.5, 26.3, 19.4 and 24.5per cent of filter paper cellulose after 7 days when used as sole carbon source.

Sushama (2005) found that, composting with water hyacinth, an aquatic weed reduced cellulose, hemicellulose, lignin and total phenol contents of coir pith after composting for 110 days. Naik (2007) explored a fungal consortium for the degradation of cellulose, hemicellulose and lignin contents in the grape vine residues and observed reduction in cellulose from 38 to 21.2 per cent, hemicelluloses, 27 to 15.37 per cent and lignin from 35 to 13.11 per cent on 90 days of composting.

El-Hanafy (2008) reported degradation of 64 and 59.2 per cent of synthetic lignins by two bacteria, *Bacillus subtilis* and *Bacillus* sp. respectively. Adejoye and Fasidi (2009) noted 92.9 per cent lignin degradation in maize cob on fermentation with the fungi *Daedalea elegans* in 90 days.

## 2.7. Microbial consortium in agro waste degradation

In nature, lignocellulosic biomass is degraded with the cooperation of many microorganisms mainly including diverse fungal and bacterial genera producing a variety of cellulolytic and hemicellulolytic enzymes under aerobic and anaerobic conditions (Kumar *et al.*, 2008). Symbiosis between cellulolytic

and non cellulolytic microorganisms has been reported to promote cellulose degradation by mixed cultures (Veal and Lynch, 1984; Pohlschroeder *et al.*, 1994; Valaskova *et al.*, 2009).

Shinde and Rote (1983) suggested the composting of sugar cane trash by different phosphate sources and microbial combinations of *Aspergillus* sp., *Penicillium* sp. and *Trichurus spirulis* by heap method and also noticed reduction in maturity period to 3 - 4 months compared to 5 months in control. Shinde *et al.* (1990) reported the production of good quality compost from sugar cane trash within four months by inoculation of mixed cultures of cellulolytic fungi supplemented with urea and super phosphate and the use of mixed cultures of different cellulolytic fungi was found to be more effective than individual fungal culture.

Potential of two microbial consortia with different strains of *B. subtilis*, *Streptomyces* sp. along with *Cellulomonas* sp. to degrade sugarcane substrate was tested *in-vitro* and both consortia showed degradation of cellulose and consortia of nine microorganisms showed better degradation (Guevara and Zambrano, 2006). Lu *et al.* (2005) noticed significant synergistic cellulose degradation with four groups of mixed cutltures of *Bacillus pasteuri, B. cereus* and bacteria belonging to the genus *Halobacillus, Aeromicrobium* and *Brevibacterium.*

Naik (2007) observed reduction in the composting period of grape vine residues to 90 days by the use of fungal consortium of *Phanerochaete chrysosporium, Trichoderma viride, Pleurotus florida* and *Aspergillus sidowia.* Balasundaram (2009) also reported the reduction in composting periods of weeds, ayurvedic herbal waste, coir pith and saw dust to 19, 22, 26 and 50 days respectively by a consortium of 14 microorganisms consisted of three fungi, two bacteria and nine actinomycetes.

Gautam *et al.* (2010) screened 14 bacterial and fungal isolates from municipal solid wastes to develop effective consortia for composting. They found three isolates, *Pseudomonas* sp., *Trichoderma viride* and *Trichoderma* sp. which

produced pectinolytic and cellulolytic enzymes at high temperatures and acidic pH were ideal for developing microbial consortium.

The potential lignocellulolytic fungi for rapid composting of rice straw were evaluated by Kausar *et al.* (2010). They tested a fungal consortium of *Aspergillus niger* and *T. viride* for the biodegradation of rice straw and observed reduction in C:N ratio from 29.3 to 19.5 in three weeks of decomposition. Wongwilaiwalin *et al.* (2010) observed effective degradation of lignocellulosic agro industrial residues such as bagasse, rice straw, corn stover and industrial eucalyptus pulp sludge by a thermophilic microbial consortium of *Clostridium* sp., *Bacillus* sp. and *Thermobacillus* sp.

Goyal and Sindhu (2011) studied composting of rice straw with three different inocula such as cattle dung, biogas slurry and consortium of three fungi (*Aspergillus awamori, Paecilomyces fusisporus* and *T. viride*). Maximum decrease in organic C (17.4%) and C:N ratio from 73.7 to 16.6 was observed in the treatment containing microbial consortium at 90 days of composting. Mohan and Ponnusamy (2011) reported composting of sugarcane trash with mixture of effective microbes (*Lactobacillus,* Yeast and *Streptomyces* sp.) isolated from fruit wastes. The trash inoculated with microbes recorded a C:N ratio of 14.4:1 as compared to 30:1 in uninoculated control at 60$^{th}$ day of composting.

Pan *et al.* (2012) explored the effect of microorganisms in the composting process of seven substrates such as common organic wastes, fruit wastes, vegetable wastes, leaves, hay, newspaper, wheat straw and rice husk with three potent bacteria, two strains of *B. subtilis* and a *Pseudomonas* sp. and the C:N ratio of all the substrates were found to reduce gradually to 25 - 30:1 within a period of 75 - 90 days.

Surtininigsih and Mariam (2012) studied the effect of cellulolytic microbial consortium of *Bacillus* sp., *Pseudomonas* sp., *Lactobacillus* sp., cellulolytic bacteria and *Trichoderma* sp. in the degradation of cow dung and rice straw in which microbial consortium degraded cow manure and rice straw in five

weeks and resulted in significant difference in water content, pH organic carbon and C: N ratio of cow manure and rice straw.

Composting of oil palm empty fruit bunches with a combination of *Streptomyces* sp., *Bacillus* sp. and *P. chrysosporium* showed reduction in composting period to 90 days indicated by the reduction in C: N ratio from 82:1 to 15:1 (Kavitha *et al.*, 2013). Mohanan *et al.* (2014) studied the efficacy of individual fungal isolates of *Trichoderma, Aspergillus and Pycnoporus* and an unidentified strain and co-cultures in delignification and saccharification of kitchen waste and *Eichhornia crassipes* and observed that, co-cultures of fungi degraded kitchen waste and *E. crassipes* after seven and four days respectively.

## 2.8. Physico – chemical characteristics of compost

### 2.8.1. Nutrient status

In general, the nutrient status of compost depends largely upon the nutrient contents of biowaste which is being composted (De- Bertodi, 1993; Wang *et.al.*, 2004).

Singh and Sharma (2002) noticed significant increase in nitrogen, phosphorus and potassium contents of wheat straw compost on pre-decomposition with a combination of bioinoculants followed by vermicomposting, with N, P and K contents of 0.98, 0.19 and 0.55 per cent respectively.

Composting of coir pith with microorganisms such as *P. sajor-caju* and *Trichoderma viride* recorded N, P and K content of 1.05, 0.05 and 1.15 per cent respectively at maturity (Kumaresan *et al.*, 2003). Preetha (2003) reported appreciably high contents of N, P and K of 3.27, 0.69, 0.70 and 3.29, 0.70, 0.70 per cent in the compost prepared from ayurvedic waste treated with *S. commune* and *P. platypus* respectively.

Sushama (2005) observed increase in the N, P and K contents of coir pith compost enriched with *Pleurotus* sp. Vermicompost prepared from cashew apple residue and leaf litter mixture showed a nutrient composition of N (1.69 %),

P (0.44%) and 0.58 per cent K (Mini *et al.*, 2005). Balasundaram (2009) recorded 3.77, 3.57, 0.64 and 0.14 per cent nitrogen in weeds, ayurvedic herbal waste, coir pith and saw dust composted with microbial consortium.

Goyal and Sindhu (2011) observed an increase in NPK status of Paddy straw from 0.65, 0.06, 0.13 to 2.09, 0.16, and 0.17 per cent on composting with fungal consortium (1%) after 90 days of composting. Nair (2011) reported that, biotic enrichment of elephant dung prior to vermicomposting significantly increased the manurial value of final compost with NPK contents of 1.34, 0.66 and 0.61 per cent respectively.

### 2.8.2. C:N ratio

C:N ratio is considered as one of the parameters for the estimation of compost maturity, stability and to define its agronomic quality. According to Krishnamurthy (1978) the satisfactory initial C:N ratio for composting is 30 - 35 and if the C:N ratio exceeds this value, it prolongs the period of composting.

Murkute *et al.* (1992) reported that, compost prepared from sugarcane trash, press mud cake, bagasse and spent mushroom substrate by microbial culture showed low C:N ratio and ranged from 13-22:1. Hart *et al.* (2002) observed a reduction in C:N ratio of fresh wheat straw from 124:1 to 44:1 on inoculation with different cellulolytic fungal isolates and 80 per cent of the isolates recorded C:N ratio below that obtained for uninoculated control (89:1).

Kumaresan *et al.* (2003) observed reduction in C:N ratio of sugarcane trash (14.8:1), coir pith (17.8:1) and press mud (12.3:1) by composting with *P. sajor-caju* and *T. viride* against 137.8:1, 75.9:1 and 14.3:1 respectively in uninoculated control treatments. Preetha (2003) reported a reduction in C:N ratio of ayurvedic compost from an initial value of 32.9 to 11.3:1 and 11.4:1 on treatment with fungi, *S. commune* and *P. platypus* respectively.

Sushama (2005) also noticed the reduction of C:N ratio of raw coir pith from 72:1 to 25:1 by composting with *Pleurotus* sp. Balasundaram (2009)

reported reduction in C:N ratio of ayurvedic herbal waste, weed waste, coir pith and saw dust on composting with microbial consortium and recorded C:N ratios of 10.31:1, 9.78:1, 18.63:1 and 275.5:1 respectively at maturity. Pan *et al.* (2012) observed that, the composting of different organic substrates with the consortium of two strains of *B. subtilis* and *a Pseudomonas* sp. showed a reduction in C:N ratio from 15:1- 128:1 to 25-30:1 at 75-90 days depending on the substrate.

### 2.8.3. pH

Changes in pH during composting is influenced by the kind of substrates and biotic agents involved. Ganapini *et al.* (1979) suggested pH as an indicator of compost maturity. Christian *et al.* (1997) reported that, composting process is most effective at pH values between 6.5 and 8.0. The decomposition of organic wastes at pH values 6.0 or below can slow down the composting process and the pH should be prevented rising above 8.5 to minimize gaseous loss of nitrogen (Pandey, 1997). Preetha (2003) noticed acidic pH values during precomposting of ayurvedic compost by the addition of bioinoculants, *S. commune* and *P. platypus.*

Mini *et al.* (2005) reported an alkaline pH of 8.9 for vermicompost produced from cashew litters and apple processed wastes. Nakasaki *et al.* (2005) also reported an alkaline pH of 8.7 at compost maturity during the thermophilic composting of organic wastes. Rajan *et al.* (2005) observed that, the pH 7.0 was optimum for the growth and lignin removal by *P. chrysosporium* in coir fibres. Saidi *et al.* (2008) opined that, an alkaline pH could enhance composting process and control pathogenic fungi, that prefer acidic growth conditions. Arunachalam and Rajasekaran (2009) noticed pH of 9.6 in coir pith composted by cyanobacteria, *Oscillatoria annae.* According to Nair (2011), elephant dung vermicompost recorded a pH of 6.63 and 6.7 respectively at maturity on pre composting with *P. platypus* and by a combination of *Aspergillus flavus and B. subtilis.*

## 2.9. Antagonism of microbial degraders

The disease suppressiveness reflects various complex interactions between pathogens and saprophytic and antagonistic microflora and between soil abiotic factors and microflora (Steinberg *et al.*, 2004). Several microorganisms have been isolated from composts and reported to suppress different soil borne diseases.

Pugliese *et al.* (2008) tested 101 microorganisms isolated from composts originated from urban and yard waste for their antagonistic activity towards soil borne plant pathogens and two organisms were found effective against *R. solani* on bean and increased the biomass of bean upto 163 per cent and two *Fusarium* isolates were effective on *F. oxysporum sp.basilici.*

## 2.10. Leaf blight of amaranth

Amaranth, the most popular leafy vegetable in Kerala is highly susceptible to leaf blight caused by *R. solani* Kuhn. The disease was first reported from Kerala by Nayar *et al.* (1996). Red varieties of amaranth having high preference in the market are more prone to the disease under humid conditions and hamper the cultivation during monsoon.

Chemical control measures recommended by Jana *et al.* (1990); KAU (1996) and Gokulapalan *et al.* (1999) have their own limitations in amaranth, being a leafy vegetable. The current trend to near-zero market tolerance for pesticides residues in fresh leafy vegetables provide an additional motivation to search for non-chemical means to control pest and diseases (Reuveni *et al.*, 2002).

### 2.10.1. The pathogen

*R. solani* was first reported from potato tubers by Kuhn in 1858. The pathogen is reported to infest 305 host species in tropics and subtropical countries (Ghaffar *et al.*, 1964). According to Das (1986), this organism is considered to be an important plant destroyer throughout the world.

Leaf blight in amaranth was first observed by Nayar *et al.* (1996). They noticed severe infection during post monsoon period and all stages of the crop were found susceptible to the disease. Symptoms of leaf blight disease of amaranth initiated as small irregular whitish cream spots on the foliage and enlarged under humidity. In the later stage, the spots became translucent with irregular brown margins and shot holes were observed in severely infected leaves leading to withering.

## 2.11. Bacterial wilt of tomato

Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*, 1995 is one of the most destructive and wide spread diseases affecting tomato in tropical and subtropical regions of the world. The first report of bacterial wilt of solanaceous crops by *Pseudomonas solanacerum* was made by Burril (1890) in connection with an unidentified bacterial disease of potato in United States. Smith (1896) described the disease, its causal agent and was the first to report bacterial wilt in potato, tomato and brinjal. The occurrence of bacterial wilt of tomato in India was first reported by Hadayathulla and Saha (1941) from West Bengal and yield loss upto cent per cent has been reported by Sadhan Kumar (1995).

Even though chemical management of the disease is found promising, the hazardous impact of agrochemicals on human health and environment and the breakdown of resistant varieties strongly demand sustainable and alternative disease management approaches.

### 2.11.1. The pathogen

Smith (1896) described the bacterium for the first time as *Bacillus solanacearum*. Later on, the pathogen was described as *P. solanaceramum* (Smith, 1914). Subsequent study of this genus based on 16S rRNA genes and polyphasic taxonomy led to the proposal of the genus, *Ralstonia* which were sufficiently distinct from other members of the genus and the wilt pathogen was finally renamed as *R. solanacearum* by Yabuuchi *et al.* (1995). The bacterium is a non spore forming, non capsulate, gram negative small rods with polar flagella.

Kelman (1954) distinguished colony variants on tetrazolium medium. The normal or wild type were irregularly round, entire, white or white with light pink centre and the mutant or butyrous type were round, translucent, smooth, deep red with a narrow light bluish margin. Phenotypic and genetic diversity of the pathogen into biotypes, varieties and races were reported by (Kelman 1953; Buddenhagen and Kelman, 1964; Hayward, 1964).

The survival and dissemination of the pathogen is generally enhanced by high soil water content. Pereira and Normando (1993) observed that, the presence of a susceptible host and high soil humidity favoured the survival of bacteria and soil type did not affect *R. solanacearum* survival.

### 2.12. Management of soil borne plant pathogens

Soil borne plant pathogens are major factors limiting the productivity of agro-ecosystem and are often difficult to control with conventional strategies. The lack of appropriate chemical control, occurrence of fungicide resistance, and break down of host resistance by pathogen populations (Mc Donald and Linde, 2002) are some of the reasons underlying the efforts to develop new disease control strategies. Moreover, the residual toxicity of fungicides in consumable plant parts also added to the search for new alternatives.

#### 2.12.1. Biological control

Interest in biological control has increased recently, fuelled by public concerns over the use of chemicals and also by the need to find alternatives to the chemicals used in disease control. Soils which are naturally suppressive to some soil borne plant pathogens such as *Fusarium oxysporum*, *Gaeumannomyces graminis*, *Pythium* and *Phytophthora* species owes its biocontrol potential to both physico- chemical and microbiological features (Whipps, 1997).

Composts and peats has been proposed both for improving soil structure (Magid *et al.*, 2001; Conklin *et al.*, 2002; Cavigelli and Thein, 2003) and also for

reducing the incidence of soil borne pathogens (Hoitink and Fahy, 1986; Lumsden *et al.*, 1983 and Schuler *et al.*, 1983).

## 2.13. Composts in plant disease management

Renewed interest in application of organic matter to soil for the control of soil borne pathogens has been stimulated by public concern about the adverse effects of soil fumigants and fungicides on the environment and the need for healthier agricultural products (Lazarovits, 2001).

A significant amount of research has been conducted on the suppression of diseases through the application of compost products worldwide. Various workers have studied the effect of composts and compost products in the management of soil borne plant pathogens. Different mechanisms are hypothesized in the disease suppressiveness by composts and most of them are the results of interactions between the antagonistic microorganisms and the pathogens either by competition, antibiosis or hyperparasitism (Hoitink *et al.*, 1993).

Several workers have suggested the disease suppression by compost products by the activation of induced systemic resistance in plants (Zhang *et al.*, 1996; Yogev *et al.*, 2010; Sang *et al.*, 2010). Zhang *et al.* (1996) observed reduction in the severity of root rot caused by *Pythium ultimum* and *P. aphanidermatum* and anthracnose by *Colletotrichum orbiculare* in the plants grown in spruce and pine bark compost than those produced in peat.

Bioassay conducted with different organics like peat mix, composted pine bark and sphagnum peat showed reduction in the severity of damping off and crown and root rot caused by *Rhizoctonia* spp. in radish and poinsettia and 15-30 per cent disease reduction was noticed in the treatments with composted pine bark (Krause *et al.*, 2001). Diab *et al.* (2003) found that, amendment of soil or container media with composts reduced the diseases caused by *Rhizoctonia* sp. upto 70 per cent. They also noticed higher levels of microbial biomass and activity which resulted in competition between compost microorganisms and *R. solani* for cellulose or other available nutrients.

Mckeller and Nelson (2003) reported the suppression of cotton damping off by *Pythium* spp. using composted leaf and twigs from deciduous trees and observed 85 per cent disease suppression with fresh compost compared to 10 year old compost.

Reduction in the symptoms of bacterial streak caused by *Pseudomonas syringae* pv. *tomato* was observed in both *Arabidopsis* and tomato plants grown in soils amended with composted forms of paper mill residual compost (PMRC) and paper mill residuals composted with bark (PMRBC) (Vallad *et al.*, 2003).The reduction in disease symptoms ranged from 34 - 65 per cent in *Arabidopsis* depending on the soil amendments whereas, tomato showed a reduction of foliar symptoms to 62 and 47 per cent respectively with PMRC and PMRBC compared to the plants grown in non amended soils.

Mathew (2004) reported the effect of coir pith compost in the reduction of bacterial wilt in solanaceous vegetables. Cheuk *et al.* (2005) investigated the disease suppression abilities of compost amendment, added to the conventional vegetable growing medium, yellow cedar saw dust. Significant reduction of Fusarium crown and root rot was observed in tomato seedlings by compost application as seed cover or plug substitute. Mikhail *et al.* (2005) noticed reduction in soil borne diseases and increased plant height in cotton seedlings with application of commercial composts.

Thirty six compost samples were analysed for physical, chemical and biological properties, including suppression of damping off caused by *P. ultimum*, *P. irregulare* and *R. solani.* Sixty seven per cent of samples with sphagnum peat moss and inorganic aggregates showed suppression of *P. irregulare* causing damping off of cucumber by 67 per cent. Sixty four per cent samples suppressed *P. ultimum* damping off and 17 per cent suppressed damping off of cabbage caused by *R. solani* (Scheuerell *et al.*, 2005).

*In vitro* evaluation of extracts of composted and vermicomposted coffee husk, coir pith and cow manure were conducted individually for the suppression

of *R. solani* of rice and after 72 h maximum inhibition (60.55%) was observed in the media amended with extracts of coir pith compost followed by extracts of coffee husk vermicompost (46.11%) (Sathianarayanan and Khan, 2008). Yadessa *et al.* (2010) reported the suppression of *R. solanacearum* of tomato by amending topsoil with three levels (1, 5 and 10%) of coco peat, farm yard manure and green manure compost. They also found an increase in the yield and the addition of higher rates of amendments was the most effective compared to 10 per cent FYM.

Castano *et al.* (2011) observed that, the composition of microorganisms in composts is affected by the chemistry of the materials from which the compost is prepared. Other researchers also opined that, composts with high lignocellulosic substances (tree barks) are mostly colonized by *Trichoderma* spp. In contrast, grape pomace, with low cellulose and high sugars gets colonized by *Penicillium* spp. *and Aspergillus* spp. (Kuter *et al.*, 1983; Gorodecki and Hadar, 1990).

Suppression of sheath blight of rice by the application of coir pith, treated with *P. sajor-caju* and *Lentinus connatus* was reported by Sudha and Lakshmanan (2011). Yang *et al.* (2012) developed an integrated approach for the control of bacterial wilt of ginger. The combination of two organic composts (maize powder and soya bean residue) and two biocontrol agents (*B. subtitis* and *B. megaterium*) showed an improvement in biocontrol efficacy of 3-30 per cent compared to individual application of bioagents. Addition of composts also increased the colonization ability of biocontrol agents and the availability of primary nutrients. They observed higher biocontrol activity (73.7%) in the combination of *B. megaterium* and maize powder compost.

Pinto *et al.* (2013) evaluated the effectiveness of composted sewage sludge incorporated into pinus bark based substrate with and without biofertilizer, fish hydrolyzate, chitozan and *T. asperellum* against Fusarium wilt in Chrysanthemum and observed significant reduction in disease severity at various intervals upto $20^{th}$ week after transplanting, which was proportionate to the level of compost added.

## 2.14. Effect of composts on plant growth promotion

Application of composted organic wastes to agricultural lands is a common practice for improving soil physical and chemical properties.

Alvarez *et al.*, 1995 studied the effect of compost on plant growth, total rhizosphere microflora and population of plant growth promoting rhizobacteria (PGPR) in the rhizosphere of tomato plants by the addition of four commercial composts and found that, three composts significantly improved plant growth. They also observed that, the variations in rhizosphere microorganisms were insignificant among the composts and the addition of composts increased the antagonistic bacterial population against *Fusarium oxysporum sp. radicis-lycopersici, Pyrenochaeta lycopersici*, *P. ultimum* and *R. solani* with an increase in siderophore producer.

Nagarajan *et al.* (1985) obtained significant increase in ground nut pod yield with combined application of NPK and coir pith (12t/ha) degraded with *Pleurotus* sp. as compared to control. Kapoor *et al.* (1990) observed that, enriched compost prepared by inoculating *Azotobacter chroococcum*, *Aspergillus awamori* and *Paecilomyces fusisporus* along with rock phosphate performed significantly better than ordinary compost in wheat. Savithri *et al.* (1993) suggested that, coir pith being rich in potash and acidity, its application will enhance the release of fixed and mineral potassium in soil and hence the quantity of potash fertilizer can be reduced in agriculture.

Decomposition of red gram stalks with *P. chrysosporium* showed reduction of composting period to one month and the composted red gram stalks applied as organic manure @ 5t/ha in soya bean resulted in significant increase in nodulation and grain yield (Jagadeesh *et al.*, 1996). In a field experiment conducted by Mamo *et al.* (1998) adequate maize grain yield was recorded in the third year of consecutive biowaste compost application and no mineral fertilizer was added. Rao (2001) suggested that coir pith composted with garden weeds, glyricidia, rock phosphate and micronutrients can be an effective component of

integrated nutrient supply system and 50 per cent of inorganic fertilizer can be saved without reduction in yield.

The feasibility of replacing peat by compost or vermicompost for the production of tomato plants in nurseries was investigated with various proportions of these substrates. Compost and vermicompost at various doses (10 and 20%) showed significant increase in aerial and root biomass of tomato plants and these treatments were also found to improve the plant growth characters (Lazcano *et al.*, 2009). Reghuvaran and Ravindranath (2010) reported that, composted coir pith mixed with nitrogen fixing bacteria is an effective potting medium for cultivation of medicinal plants. Espiritu (2011) observed that, biocompost prepared from coconut coir dust - poultry manure mixture inoculated with N- fixing bacteria, *Azotobacter* sp. and cellulolytic fungi, *T. harzianum* , applied in potted mung bean (5g/pot) recorded highest plant biomass and number of nodules after 30 days.

Mrabet *et al.* (2012) studied the agronomic value of compost on maize and lettuce crops and found that, adequate nutrient contents of compost improved yields of both crops which are proportionally related to the dose of compost. Namasivayam and Bharani (2012) evaluated the growth promotion effect of mung bean with fruit wastes composted with effective microbes, which showed enhancement in growth parameters and reduction in pests and diseases.

# *Materials and Methods*

# 3. MATERIALS AND METHODS

The present study on "Efficacy of ligno-phenolic compost in the suppression of soil borne plant pathogens" was conducted in the Department of Plant Pathology during 2011 – 2014. Composting experiments and sample analysis were carried out in the Department of Soil Science and Agricultural Chemistry and the field experiments were conducted in the research plots of College of Horticulture, Vellanikkara. Various lignin-tannin rich agrowastes selected for the study were ayurvedic waste, coir pith and leaf litters of cashew, teak and mango.

## 3.1. COLLECTION OF SAMPLES

Samples of both soil and partially decomposed substrates were collected from the dumping sites of ayurvedic wastes, coir pith, elephant dung, cashew and teak plantations, mango orchards, forest areas, coconut basins mulched with coconut by products from 15 sources and 34 locations throughout Kerala and border areas of Tamil Nadu as listed in Table 1 (Plate 1). *Kashayam* and *arishtam* formulation wastes were collected separately from the ayurvedic units wherever it was possible. Samples were collected from three different sites of each location and then pooled. These samples were stored in sealed poly bags under refrigerated condition for the isolation of microbial degraders.

## 3.2. ISOLATION OF MICROBIAL DEGRADERS

Both enriched and non enriched samples were used for the isolation of microbial degraders.

### 3.2.1. Enrichment of samples

Twenty five grams of representative samples were taken in 250 ml conical flasks and were enriched with 0.5 g of selective nutrients *viz.* cellulose powder,

## Table 1. Locations for sample collection

| Sl. No. | Source | Location |
|---|---|---|
| 1 | Ayurvedic pharmaceutical units | |
| | a) SD pharmacy | Alappuzha |
| | b) Kerala Ayurveda Ltd | Angamaly, Ernakulam |
| | c) Kottakkal Aryavaidhyashala | Malappuram |
| | d) Oushadhi | Kuttanellur, Thrissur |
| | e) Vaidyaratnam | Ollur, Thrissur |
| 2 | Coir retting areas | Thanneermukkam, Alappuzha |
| 3 | Coir fibre extraction areas | Kalavoor, Alappuzha |
| | | Gopalapuram, Palakkad |
| | | Unchavelampatty,Pollachi, Tamil Nadu (TN) |
| 4 | Coir pith dumping sites | Adivaram, Kozhikode |
| | | Andiyoor, Pollachi, TN |
| | | Kolarpatty, Pollachi, TN |
| 5 | Coconut basins mulched with coconut by products | Ollukkara, Thrissur; |
| | | Thimmankuth, Pollachi, TN |
| 6 | Elephant dung dumping site | |
| | a. Anakotta | Punnathurkotta Guruvayur, |
| | b. Anaparambu | Padukad, Thrissur |
| 7 | Forest areas | Dhoni, Palakkad |
| | | Poonjar, Kottayam |
| | | Silent valley, Palakkad |
| | | Chimmony, Thrissur |

| | | |
|---|---|---|
| 8 | Cashew plantations | Madakkathara, Thrissur |
| 9 | Teak plantations | Peechi, Thrissur |
| | | Nilambur, Malappuram |
| 10 | Mango orchards | Vellanikkara, Thrissur |
| | | Ollukkara, Thrissur |
| | | Ozhalapathy, Palakkad |
| 11 | Compost pits | Composting unit,Vellanikkara |
| | | Vermicomposting unit, Vellanikkara |
| 12 | Spent Mushroom Substrates dumping sites (straw & saw dust) | Mushroom production unit, Vellanikkara |
| 13 | Saw mill | Paravattani, Thrissur |
| 14 | Termite mound | Ollukkara, Thrissur |
| 15 | Fresh cow dung | Vellanikkara, Thrissur |

# PLATE -1

## COLLECTION OF SAMPLES FROM VARIOUS LOCATIONS

**Ayurvedic pharmaceutical unit (Kottakkal)**

**Coir fibre extraction area (Pollachi)**

**Coir pith dumping site (Adivaram)**

**Elephant dung dumping site (Guruvayoor)**

**Coconut by product mulched basin (Ollukkara)**

**Teak plantation (Nilambur)**

lignin sulphonate and tannic acid for the isolation of maximum potential degraders. The enriched samples were incubated at room temperature, 28 ± 2°C, for five days.

### 3.2.2. Standardization of dilution factor for isolation

Microbial degraders were isolated from various samples by serial dilution agar plate technique (Johnson and Curl, 1972). Dilution factors for the isolations of degraders were standardized for both enriched and non enriched samples. Dilutions upto $10^{-9}$ and $10^{-6}$ were tried for cellulose and lignin/tannin degraders respectively for both samples.

### 3.2.3. Isolation of selected microbial degraders

Microbial degraders of cellulose, lignin and tannin were isolated from enriched and non enriched samples using the standardised dilutions on Dubo's medium, (Deokar and Sawant (2004), lignin sulphonate (Thimmaiah, 1989) and tannic acid media (Thormann *et al.*, 2002) respectively by pour plate method. (The compositions of media are detailed in Appendix I). The inoculated plates were incubated at room temperature for three days. Three replications were maintained for each sample. Observations on microbial population with distinct clear zone were recorded at three days after incubation (DAI). Degraders were quantified separately as fungi, bacteria and actinomycetes. Single colony of degraders with good clear zone was selected, purified and subcultured on selective media slants and maintained at $4^0$C for further studies.

The isolates were numbered representing the source of sample, component degraded, type of organism and the Arabic numerals in the serial order.

## 3.3. *IN VITRO* SCREENING OF MICROBIAL DEGRADERS ON SELECTIVE MEDIUM

The isolated degraders were tested individually on appropriate selective media for their degrading ability of selective nutrient adopting agar plate diffusion assay.

### 3.3.1. *In vitro* screening of cellulose degraders

The cellulolytic activity of isolated microbes was tested *in vitro* as suggested by Kasana *et al.* (2008). Eight mm discs of seven day old cultures of fungi and actinomycetes were placed at the centre of carboxy methyl cellulose agar (CMC) mediated plates (Guevara and Zambrano, 2006) (Appendix I). Likewise, 48 h old bacterial cultures were spot inoculated on the same medium. In case of dry spore forming fungi, spore suspension was prepared with 8 mm disc of fungal cultures in 1 ml sterile water and 50 µl of this suspension was poured into agar wells made with 8mm cork borer at the centre of the mediated plates. Inoculated plates were incubated at $28 \pm 2^0$C in duplicates and flooded with Gram's iodine on 2 and 3 DAI for bacteria and fungi/actinomycetes respectively. Observations on the diameter of diffusion zones were recorded and the degrading ability was assessed using 1-5 scale score chart, which is mentioned below.

| Score | Diameter of diffusion zone (cm) |
|---|---|
| 1 | < 1 |
| 2 | 1- 2 |
| 3 | 2- 3 |
| 4 | 3- 4 |
| 5 | > 4 |

### 3.3.2. *In vitro* screening of lignin and tannin degraders on selective media

Lignin and tannin degrading potential of selected microbes were tested on lignin sulphonate and tannic acid media respectively. Fungi, actinomycetes and bacteria were inoculated on the media as mentioned above. Inoculated plates were

incubated at room temperature with two replications and observations on diffusion zones were recorded at 2 and 3 DAI.

## 3.4. CROSS DEGRADABILITY OF SELECTED MICROBIAL DEGRADERS

Efficiency of selected microbes to degrade all the three chemical components *viz.* cellulose, lignin and tannin were cross tested on the selective media.

Twelve cellulose degraders selected from the above study were tested on lignin and tannin selective media for their degrading potential. Likewise, 18 lignin degraders were tested on CMC and tannin selective media and five tannin degraders on CMC and lignin selective media. Two replications were maintained for each degrader. Observations on diameter of diffusion zones were recorded at 2 and 3 DAI.

## 3.5. *IN VITRO* SCREENING OF SELECTED DEGRADERS ON HOST SUBSTRATES

Thirty three degraders were selected based on the size of diffusion zone, type and species of microorganism and tested for the efficacy to degrade their respective host substrates. The degraders selected for the study are listed in Table 2.

The substrates used for this study were ayurvedic waste, coir pith, elephant dung, leaf litters of cashew, teak and mango and paddy straw. The experiment was carried out separately in 500 ml conical flasks with substrates @ 25 -100 g and in plastic pots of size, 8" with substrates @ 50 -200g depending on the volume of substrate. Microbial inoculum was prepared by incubating the degraders separately in 100 ml potato dextrose broth @ one culture disc of 1cm size/100ml medium and incubated seven days for fungi/actinomycetes and two days for bacteria. The substrates were inoculated separately with undiluted inoculum @ 200 ml/kg substrate (ayurvedic waste & coir pith) and 300 ml/kg substrate (leaf litters & paddy straw) and

**Table 2. Microbial degraders selected for *in vitro* screening on host substrates**

| Sl. No. | Type of organism | Degraders | Source |
|---|---|---|---|
| 1 | Fungi | ACF-6, ALF-20, ALF-26, ALF-30 & ATF-19 | Ayurvedic waste |
| | Bacteria | ACB- 2 & ALB-2 | |
| | Actinomycetes | ALA-1,ACA-5 | |
| | Yeast | ATY- 1 | |
| 2. | Fungi | CCF-4,CLF-1,CLF-6,CLF-12, CLF-13,CLF-16, CLF-22, CLF-36 & CTF-4 | Coir pith |
| | Bacteria | CCB-8 | |
| 3. | Fungi | ECF-2 | Elephant dung |
| | Actinomycete | ECA-8 | |
| 4 | Fungi | TCF-5, TLF-8, MLF-1, FTF-11, CaLF-5, CaLF-7 & CaTF-7 | Teak, Mango, Cashew & forest leaf litters |
| | Actinomycetes | CaCA-3 & CaCA-5 | |
| 5 | Bacteria | CdCB-1 | Fresh cow dung |
| 6 | Actinomycete | TmCA -2 | Termite mound |

moisture level adjusted to 50 per cent (ayurvedic waste & coir pith) to 60 per cent (leaf litters & paddy straw) with sterile water. Plastic pots were covered with moistened cloth. Moisture was maintained and the substrates were turned daily for one week and later thrice a week till maturity. All treatments were replicated thrice. Visual observation on texture was recorded to assess compost maturity.

### 3.6. *IN VIVO* EVALAUATION OF SELECTED DEGRADERS ON RESPECTIVE HOST SUBSTRATES

The efficiency of degraders on respective host substrates were evaluated under *in vivo* conditions using 16" sized earthen pots. Twenty two potential degraders selected from the above *in vitro* experiments were used for this study. Substrates, mass multiplication of microbial inoculum and moisture level maintained were same as that followed in *in vitro* studies. One to five kilograms of substrates, were filled in the earthen pots depending on their volume and inoculated with 25 per cent microbial inoculum @ 200-300ml/kg depending on the substrate and covered with moistened cloth. Inoculated substrates were turned regularly for first seven days and later thrice a week. Required moisture level was maintained by occasional sprinkling of water during the period of composting. The experiment was laid out in completely randomised design (CRD) with two replications. Substrates were examined regularly and visual observation on texture was recorded.

### 3.7. *IN VIVO* EVALUATION OF SELECTED DEGRADERS ON OTHER HOST SUBSTRATES

The degraders tested *in vivo* on their host substrates were further cross tested on other substrates to evaluate their efficiency to degrade any ligno-phenolic plant residues other than their respective hosts.

This study was also carried out in big earthen pots as mentioned above with same quantity of substrates, inoculum rate and concentration. The moisture level was maintained at 50 - 60 per cent and turning was done thrice a week for aeration. Of the

substrates used in the earlier study, paddy straw was substituted with fodder grass. Observations to assess compost maturity were recorded regularly.

## 3.8. STUDIES ON MUTUAL COMPATIBILITY OF SELECTED DEGRADERS

Eighteen promising degraders (12 fungi, two bacteria, three actinomycetes and one yeast) selected from *in vivo* studies, were tested for their mutual compatibility *in vitro* using potato dextrose agar medium (PDA medium) detailed in Appendix I.

### 3.8.1. Compatibility between fungi

The interactions between fungal degraders were studied by dual culture technique. Eight mm mycelial discs of two fungal degraders were placed on PDA mediated plates at equidistance of 2 cm from the periphery. Plates were kept in triplicates for each combination and incubated at room temperature to observe compatibility reaction.

### 3.8.2. Compatibility between fungi and bacteria/actinomycetes/ yeast

Spore suspensions of four different fungal degraders were spotted separately at the four corners on the bacteria / actinomycete / yeast seeded mediated plates at equidistance of 2cm from the periphery. All the combinations were kept in triplicates. Observations on inhibition zones were recorded at 2 DAI.

### 3.8.3. Compatibilty between bacteria with bacteria/ actinomycetes/ yeast

Mutual compatibility between bacteria, actinomycetes and yeast were studied by cross streaking method. Three bacterial degraders were streaked horizontally and the actinomycetes /yeast vertically on PDA medium and observed for lysis at the junctures. Each combination was triplicated.

## 3. 9. IDENTIFICATION OF MICROBIAL DEGRADERS

Microbial degraders selected for development of consortium were identified based on cultural and morphological characters. Biochemical and molecular characterisation were also carried out for bacterial isolates.

### 3.9.1. Identification of fungi and actinomycetes degraders

The fungal and actinomycete degraders found most effective *in vitro* and *in vivo* were identified based on cultural and morphological characters. The morphological characters were studied by slide culture technique. The cultural characters like colour, growth and texture of the colonies and morphological characters like type of mycelium, size and shape of conidiophores and phialides, colour, shape and size of conidia were also recorded. Based on this, the degraders were tentatively identified at genus level and for further confirmation and species level identification, cultures were sent to National Centre for Fungal Taxonomy, New Delhi and National Fungal Culture Collection Of India, Agharkar Research Institute, Pune.

### 3.9.2. Identification of bacterial degraders

Selected bacterial degraders were characterized based on cultural, morphological, biochemical and molecular characters following the methods suggested in Manual of Microbiological Methods, published by the society of American Bacteriologist (1957) and also by the Bergy's Manual of Systematic Bacteriology, Vol - I (Stanley *et al.*, 1989). The cultural characters like colony margins, elevation, colour etc., morphological (Gram's reaction, shape) and biochemical characters of the isolates (catalase reaction, denitrification, starch hydrolysis, gelatin liquefaction, citrate utilization, lysine utilization, ornithine utilization, urease detection, phenyl alanine deamination, nitrate reduction, hydrogen sulphide production, utilization of sugars and alcohols – glucose, sucrose, lactose,

arabinose, adonitol and sorbitol ) were studied. The Hi Assorted TM Biochemical test kit for Gram negative rods were also employed for characterization of the isolates. The bacterial isolates were subjected to molecular characterisation by 16S rRNA sequencing to identify them upto the species level. The BlastN programme (http:// www.ncbi.nlm.nhm.gov/blast) was used to find out the homology of nucleotide sequences.

## 3.10. DEVELOPMENT OF MICROBIAL CONSORTIUM

Based on the ability to degrade all the three chemical components, early maturity of composting, type and species of microorganisms and mutual compatibility, 13 microbial degraders were selected for the formulation of consortia. Two microbial consortia, I and II were developed with 10 degraders each, and consortium –I consisted of five fungi, of which two were non human pathogenic *Aspergillus* spp., two bacteria, two actinomycetes and yeast. Consortium-II included, five fungi, two bacteria and three actinomycetes.

## 3.11. PREPARATION OF MICROBIAL CONSORTIUM

Microbial degraders were mass multiplied in potato dextrose broth. Three one cm sized discs of seven day old fungi and actinomycetes and three loopful of 48 h old bacteria were inoculated separately in 300 ml potato dextrose broth and incubated for seven days for fungi and actinomycetes and 2 days for bacteria. The bacterial incubation was adjusted, in order to complete the incubation periods of all degraders on the same day. After required period of incubation, the mass multiplied, 10 degraders were mixed together to make three litre inoculum, which was diluted to the desired concentration. This method was followed for the preparation of consortial inoculum in all other related experiments.

## 3.12. *IN VITRO* SCREENING OF MICROBIAL CONSORTIUM

Microbial consortia - I and II were screened *in vitro* for their efficacy to degrade ligno-phenolic substrates *viz.* ayurvedic waste, coir pith, leaf litters of cashew, teak and mango (1:1:1) and mixture of ayurvedic waste, coir pith and leaf litters (1:1:1) along with cow dung slurry and uninoculated substrates as control. Microbial consortium and cow dung slurry of 20 per cent concentration were inoculated separately on known quantity of substrates (200ml/kg of substrate) in 500 ml conical flasks (ayurvedic waste, coir pith and elephant dung (50 g), leaf bits of cashew, teak and mango @ 25g and mixture of substrates @ 50g). Moisture content was maintained at 50 - 60 per cent depending on the substrates. The treatments were replicated thrice. Turning and watering were done at periodical intervals and observation on compost maturity was recorded regularly.

## 3.13. *IN VIVO* SCREENING OF MICROBIAL CONSORTIUM

The consortium selected from *in vitro* study was further evaluated under *in vivo* condition on the same substrates in big earthen pots (16") along with cow dung slurry and uninoculated control as check. Quantity of substrates used for composting varied from 1- 5 kg depending on the volume of the substrate. Microbial consortium and cow dung slurry at a concentration of 20 per cent were inoculated separately to the substrates @ 200ml/kg substrate. Three replications were maintained for each treatment. Periodical turning and maintenance of moisture was carried out thrice a week. Observations on texture were recorded regularly to assess compost maturity.

## 3.14. LARGE SCALE COMPOSTING OF LIGNO-PHENOLIC SUBSTRATES WITH MICROBIAL CONSORTIUM

A large scale composting experiment of lignin-tannin rich agrowastes using microbial consortium was conducted in concrete tanks of size 2x 2' in the composting shed of Department of Soil Science and Agricultural Chemistry during April- May.

Microbial consortium was tested with large quantity of substrates, varied from 15-75 kg depending on the volume of the substrate ie. ayurvedic waste (75 kg), coir pith (50 kg), leaf litters of teak, cashew and mango @ 1:1:1 (15 kg) and mixture of all substrates (50 kg). Since, cow dung slurry @ 20 per cent concentration is used for normal aerobic composting, the concentration of microbial consortium was also adjusted to 20 per cent. Substrates were inoculated with microbial consortium alone, cow dung slurry alone and a combination of both @ 200ml/kg substrate. Substrates were stacked in four layers and the inoculum was applied uniformly on each layer with thorough mixing. Moisture content of substrates was adjusted to 50 - 60 per cent. Tanks were then covered with moistened gunny bags. Turning and moistening of the pile were carried out thrice a week. The treatments were replicated thrice for each substrate. An aerobic composting of ordinary plant residues with 20 per cent cow dung slurry and uninoculated substrates served as control.

### 3.14.1. Observations

The variations in temperature were noted using multichannel temperature recorder at 11 am daily during entire period of composting (Thomas, 2001). pH was recorded at fortnightly intervals using pH pen. Qualitative observations on texture and colour were recorded periodically to judge compost maturity. Number of days required for completion of the composting was also recorded. Maturity period was assessed based on temperature, texture and C: N ratio.

## 3.15. COMPATIBILITY OF SELECTED DEGRADERS WITH COW DUNG

The combined application of microbial consortium and cow dung slurry showed longer period of composting as compared to individual applications. Hence a compatibility study was carried out by inoculating the selected degraders on PDA medium amended with 20 per cent cow dung slurry/ supernatant. Observation on compatibility reactions of microbial degraders with microbes of cow dung was recorded.

## 3.16. REISOLATION OF MICROBIAL DEGRADERS FROM MATURE COMPOSTS

The survival of inoculated degraders at compost maturity was confirmed by the reisolation of microbial degraders from final compost products on respective selective media adopting standardized dilutions employed for isolation of various degraders.

## 3.17. ESTIMATION OF C:N RATIO AND NUTRIENT CONTENTS OF SUBSTRATES AND MATURE COMPOSTS

Various substrates were analysed for C: N ratio, macro and micronutrient *viz.* nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), zinc (Zn), iron (Fe) and organic carbon (C) before and after composting as per standard protocols (FAI, 2007; Jackson,1958; Perkin - Elmer, 1982).

## 3.18. ESTIMATION OF HEAVY METAL CONTENT IN VARIOUS COMPOST SAMPLES

The presence of heavy metals *viz.* cadmium, chromium, lead, arsenic, mercury and nickel were analysed as per the methods described in FAI (2007).

## 3.19. ESTIMATION OF BIOCHEMICAL COMPONENTS OF SUBSTRATES AND MATURE COMPOST

Biochemical analysis for cellulose, lignin and tannin contents of substrates as well as final compost products were carried out as per the protocols outlined by Updegroff, 1969; Thimmaiah, 1989 and Sadasivam and Manickam, 1997 respectively.

## 3.20. PREPARATION OF TALC BASED CONSORTIAL FORMULATION

For the development of talc based consortial formulation, degraders were inoculated separately in 100 ml potato dextrose broth @ 1 cm disc of seven day old fungi and actinomycetes and one loopful of 48 h old bacteria. Inoculated broths were incubated for seven days for fungi and actinomycetes and 2 days for bacteria. After incubation broths were mixed together, to make one litre of inoculum and added to sterile talc @ 300 ml/kg under aseptic conditions, mixed well, dried, packed in polythene bags and stored till use.

## 3.21. COMPOSTING OF VARIOUS SUBSTRATES WITH TALC BASED CONSORTIAL FORMULATION

Composting experiments were carried out in big concrete tanks during the months of April - May and October - November with large quantity of substrates, varied from 15 - 50 kg depending on the volume of substrate ie. ayurvedic waste (50 kg) coir pith (50 kg), leaf litters of teak, cashew and mango @ 1:1:1 (15 kg) and mixture of all substrates (50kg). Substrates were inoculated with talc based formulation @1kg/100kg substrate. Substrates were stacked in four layers and equal quantity of microbial inoculum was spreaded uniformly on each layer with thorough mixing. Moisture content of substrates was adjusted to 50 - 60 per cent. Tanks were

then covered with moistened gunny bags. Turning and moistening of the pile were carried out thrice a week. The treatments were replicated thrice for each substrate.

## 3. 22. SCREENING FOR THERMOTOLERANCE POTENTIAL OF MICROBIAL DEGRADERS

The heat tolerant ability of degraders selected for the microbial consortium was tested *in vitro*. A loop full of 48 h old bacterial and 5 mm mycelial discs of seven day old cultures of fungi and actinomycetes were inoculated separately to 5 ml potato dextrose broth in glass vials. Inoculated vials were incubated in BOD at specific temperatures of 40°C, 45°C, 50°C, 55°C, 60°C and 65°C. The viability of the cultures was tested on PDA medium two days after incubation for bacteria and four days after incubation for fungi and actinomycete.

## 3.23. EXTRACELLULAR ENZYME ACTIVITIES OF SELECTED DEGRADERS

The extra cellular enzyme activities of selected degraders were studied *in vitro* adopting the protocols suggested by various workers.

### 3.23.1. Catalase

A few drops of three per cent hydrogen peroxide were added at the centre of a sterile glass slide. A loopful of bacterial culture was then mixed with hydrogen peroxide (Cappucino and Sherman, 1992) and observed for effervescence.

### 3.23.2. Cellulase

Efficiency of degraders to produce cellulase/β-1, 4 endoglucanase was tested on CMC agar medium (Kasana *et al.*, 2008). The isolates were spotted on the medium and incubated for 2 days for bacteria and 7 days for fungi and actinomycetes. The incubated plates were then flooded with Gram's iodine and observed for the presence of clear zone around the colony.

### 3.23.3. Amylase

Selected degraders were tested for their ability to hydrolyze starch on Czapex-Dox agar medium (Appendix I). Degraders were spot inoculated/streaked on the medium in Petri dishes and incubated at room temperature for 5 days. After incubation the plates were flooded with Lugol's iodine and observed for the appearance of clear zones around the growth of colony (Aneja, 2003).

### 3.23.4. Xylanase

Xylanase enzyme production by the degraders was tested by inoculating the cultures on xylan agar medium (Appendix I). Inoculated plates were flooded with one per cent congo red solution for 10 min and then destained with 1N NaOH solution. Appearance of clear zone the around the colonies was observed (Cai *et al.*, 1994).

### 3.23.5. Protease

The protease activity of the degraders was tested on one per cent skimmed milk agar medium (Appendix I). The production of clear zone around the colony growth on overnight incubation was recorded (Singh and Saxena, 2011).

### 3.23.6. Polyphenol oxidase (PPO)

The presence of polyphenol oxidase was tested using tannic acid medium as out lined by Kausar *et al.* (2010). Mediated plates were inoculated at the centre with 5mm mycelial discs of five day old cultures of fungi, a loop full of bacteria and actinomycetes. Inoculated Petri dishes were incubated at the room temperature for four days. Formation of dark brown pigmentation around the growth of the organism was observed and recorded.

### 3. 23.7. Lignin Peroxidase

Lignin peroxidase activity of the degraders was tested by the protocol suggested by Kausar *et al.* (2011). Degraders were inoculated on the media and incubated at room temperature. Growth of the organism on the selective media was recorded.

## 3.24. DISEASE MANAGEMENT

The antagonistic activity of the selected degraders against major soil borne pathogens and the efficacy of various composts in the management of two important diseases of vegetables in Kerala was also studied.

### 3.24.1. Isolation of pathogens

Two soil borne pathogens tested in the study were isolated from the infected plants parts following standard protocols.

#### 3.24.1.1. *Isolation of Rhizoctonia solani*

Amaranth leaves infected with leaf blight disease were collected from the fields of Department of Olericulture, College of Horticulture, Vellanikkara. Infected leaves were brought to the laboratory, washed under tap water and dried with blotting paper. Infected portions along with healthy portions were cut into smaller bits of 5 mm size and surface sterilized with one per cent sodium hypochlorite for 1 min and washed with three changes of sterile water. The bits were transferred aseptically to sterile Petri dishes containing potato dextrose agar medium and dishes were incubated at room temperature. The fungus was purified by hyphal tip method and pure culture was maintained on PDA slants at 4°C for further studies.

### 3.24.1.2. *Isolation of Ralstonia solanacearum*

Wilted tomato plants collected from the field of Department of Olericulture were brought to the laboratory. The plants were washed under tap water, root portions were removed, and subjected to ooze test. An oblique cut was made at the basal stem portion and the cut end was placed in a test tube containing sterile water and observed for stream of bacterial ooze from the cut end to confirm the association of bacterial pathogen with the wilt disease. The plants which showed streaming of ooze from the cut ends were used for isolation of pathogen. Stem portion of the infected plant was cut into small segments of 1 cm and the bits were surface sterilized adopting standard protocol. Surface sterilized bits were placed on a sterile glass slide with a drop of sterile water and teased apart. A loopful of this suspension was streaked on Triphenyl Tetrazolium Chloride (TZC) medium (Appendix I) in Petri dish and the plates were incubated at room temperature for 48 h. The typical white colonies with pinkish centre were selected, purified and maintained in glass vials containing sterile water and kept in refrigerator for further studies.

### 3.24.2. *In vitro* evaluation of selected degraders against soil borne plant pathogens

The antagonistic potential of microbial degraders selected for consortium were evaluated against five important soil borne fungi and bacterial wilt pathogen, *R. solanacearum* under *in vitro* condition.

### 3.24.2.1. *In vitro screening of selected degraders against fungal pathogens*

Antagonistic activity of 12 microbial degraders were screened against the major soil borne fungi, *viz. Pythium* sp., *Phytophthora* sp., *Rhizoctonia* sp., *Fusarium* sp. and *Sclerotium* sp. Two methods were adopted for testing antagonism. Simultaneous antagonism was adopted in which both pathogen and degraders are

either slow growers or fast growers and deferred antagonism was adopted in which either the pathogen or the degrader is fast grower.

**a. *Simultaneous antagonism***

Simultaneous antagonism method was adopted for slow growing pathogens, *viz. Fusarium* sp. and *Phytophthora* sp. and all degraders except *Trichoderma* sp. Eight mm mycelial discs of seven day old culture of pathogen as well as degraders were placed on PDA mediated plates 2 cm distance from the periphery. The inoculated plates in triplicates were incubated at room temperature. Observations on radial growth of pathogens were recorded. Controls without antagonists were maintained for each treatment.

**b. *Deferred antagonism***

Antagonism of selected degraders except, *Trichoderma* sp. was also tested against fast growing pathogens *viz. Pythium* sp., *Rhizoctonia* sp. and *Sclerotium* sp. adopting deferred antagonism method. Sterilised Petri dishes containing PDA medium were inoculated with 8 mm mycelial discs of seven day old cultures of pathogens as well as degraders at equal distance of 2 cm from the periphery. Monocultures of pathogens served as control. Three replications were kept for each treatment and were incubated at room temperature. The observations on radial growth of pathogens were recorded.

Per cent inhibition of pathogen was calculated using the formula suggested by Vincent (1927).

$$\text{Per cent inhibition} = \frac{C-T}{C} \times 100$$

Where C- Radial growth of pathogen in control plate (cm)

T - Radial growth of pathogen in dual culture (cm)

#### 3.24.2.2. *In vitro evaluation of degraders against R. solanacearum*

The antagonistic effect of selected degraders against *R. solanacearum* was studied *in vitro* condition adopting dual culture method (Dennis and Webster, 1971).

Fungal degrader suspensions prepared as mentioned in 3.3.1 were placed in 8 mm diameter agar wells prepared at the centre of the PDA plates seeded with 48 h old culture of *R. solanacearum.* Bacterial and yeast degraders were spotted at the centre of the bacterial lawn. Three replications were maintained for each degrader. The plates with pathogen alone served as control. Petri dishes were incubated at room temperature and observed for the inhibition of pathogen. Diameter of inhibition zones were measured after 48 h and per cent inhibition was calculated.

## 3.25. FIELD EVALUATION OF VARIOUS COMPOSTS ON THE MANAGEMENT OF LEAF BLIGHT OF AMARANTH AND BACTERIAL WILT OF TOMATO

Two field experiments were conducted to study the efficacy of various ligno-phenolic composts on the management of leaf blight of amaranth and bacterial wilt of tomato.

### 3.25.1. Efficacy of various compost on the management of leaf blight of amaranth

A field experiment was laid out in June- August, 2013 to study the efficacy of various ligno-phenolic compost products on the management of leaf blight of amaranth caused by *Rhizoctonia solani.* Composts and cow dung were applied @ 50 t/ha as full basal or in splits. NPK fertilizers and bioagent, *Pseudomonas fluorescens* were also included for comparison. The details of the experiment are as follows.

| | |
|---|---|
| Crop | : Amaranth |
| Variety | : Arun |
| Design | : RBD |

| | |
|---|---|
| Replications | : 3 |
| Plot size | : 2.5 x 2.0 m |
| Spacing | : 30 x 30 cm |
| No. of. treatments | : 15 |
| No. of plants/ treatment | : 24 |

### 3.25.1.1. *Treatments details*

T1- Ayurvedic compost - full basal

T2- Ayurvedic compost - ½ basal+ ½ top dressing, 30 days after planting (DAP)

T3- Coir pith compost - full basal

T4- Coir pith compost - ½ basal + ½ 30 DAP

T5- Leaf litter compost - full basal

T6- Leaf litter compost - ½ basal + ½ 30 DAP

T7- Mixture compost- full basal

T8- Mixture Compost -½ basal + ½ 30 DAP

T9- Ordinary Compost – full basal

T10- Ordinary compost - ½ basal + ½ 30 DAP

T11- Cow dung @ 50 t/ha - full basal

T12- Cow dung- ½ basal + ½ 30 DAP

T13- *Pseudomonas fluorescens* – 2 per cent – ATP + 30 DAP

T14- NPK fertilizers as per POP @ 50:50:50 kg/ha - ½ basal + ½ 30 DAP

T15- Control (with out treatment)

### 3.25.1.2. *Nursery of amaranth*

Nursery of amaranth was raised in earthen pots with sterilized potting mixture consisted of soil, sand and cow dung @1:1:1, using the variety, Arun. Seedlings were kept in net house and watered regularly.

### 3.25.1.3. *Preparation of field and transplanting*

Plots were prepared by ploughing followed by levelling. Shallow trenches were taken with length of 2.5 m and width of 30 cm. Composts/cow dung were applied in the trenches at specified rates and mixed thoroughly. NPK fertilizers were applied as urea, rajphos and muriate of potash @ 109, 250 and 83kg /ha and 2 per cent *P. fluorescens* suspension (20g/l) was applied as soil drenching at the time of planting and 30 days after planting @ 6 l/$m^2$. Twenty day old seedlings were transplanted in the treated trenches at a spacing of 30 cm between the plants and 30 cm between the rows.

### 3.25.1.4. *Observations*

Fifteen plants were randomly selected from each treatment and labelled for taking observations. Observations on plant height, disease severity and yield were recorded.

### 3.25.1.5. *Disease incidence*

Disease incidence was observed in all plants at 30 and 60 days after planting (DAP). Per cent disease incidence was calculated using the formula:

$$\text{Per cent disease incidence} = \frac{\text{No.of plants infected}}{\text{Total no.of plants observed}} \times 100$$

### 3.25.1.6. *Disease severity*

Disease severity was recorded from the 15 labelled plants in each treatment Ten leaves were randomly selected from each plant and scoring was done using 0 - 9 scale score chart (Uppala, 2007).

| Grade | Description |
|---|---|
| 0 | No symptom |
| 1 | < 10 % leaf area infected |
| 3 | > 10-25 % leaf area infected |
| 5 | > 25-50 % leaf area infected |
| 7 | > 50-75 % leaf area infected |
| 9 | > 75 % leaf area infected |

Per cent disease severity was calculated using the formula suggested by Wheeler (1969).

$$\text{Per cent disease severity} = \frac{\text{Sum of all numerical ratings}}{\text{Total no.of leaves observed x maximum disease grade}} \times 100$$

### 3.25.2. Efficacy of various composts on the management of bacterial wilt of tomato

A field experiment was conducted to study the efficacy of ligno-phenolic composts in the management of bacterial wilt of tomato caused by *R. solanacearum*. The study was conducted during Oct. 2013 - Jan. 2014 in the wilt sick plots. Composts and cow dung were applied @ 25 t/ha as full basal or in two splits. NPK fertilizers and chemical fungicide were also included for comparison. The details of the experiment are as follows.

#### 3.25.2.1. *Experiment details*:

Crop : Tomato

Variety : Mukthi

Design : RBD

Replications : 3

Plot size : 2.7 x 2.4 m

Spacing : 60 x 45 cm

No. of. treatments : 15

No. of plants/ treatment : 24

### 3.25.2.2. *Treatments details*

T1- Ayurvedic compost - full basal

T2- Ayurvedic compost - ½ basal+ ½ top dressing, 30 days after planting (DAP)

T3- Coir pith compost - full basal

T4- Coir pith compost - ½ basal + ½ 30 DAP

T5- Leaf litter compost - full basal

T6- Leaf litter compost - ½ basal + ½ 30 DAP

T7- Mixture compost- full basal

T8- Mixture Compost -½ basal + ½ 30 DAP

T9- Ordinary Compost – full basal

T10- Ordinary compost - ½ basal + ½ 30 DAP

T11- Cow dung @ 25 t/ha - full basal

T12- Cow dung - ½ basal + ½ 30 DAP

T13- Copper hydroxide – 2g/l – ATP + 30 DAP

T14- NPK fertilizers as per POP @ 75:40:25 kg/ha - ½ basal + ½ 30 DAP

T15- Control (with out treatment)

### 3.25.2.3. *Nursery*

Seedlings of tomato variety, Mukthi were raised in earthen pots containing sterilised potting mixture consisted of soil, sand and cow dung @1:1:1.

### 3.25.2.4. *Preparation of field and transplanting*

Experimental plots were prepared by ploughing followed by levelling. Shallow trenches were taken at a length of 2.7 m and width of 30 cm and NPK fertilizers were applied as urea, rajphos and muriate of potash @ 163, 200 and 42 kg/ha. Soil drenching of copper hydroxide (2g/l) was given at the time of planting and 30 days after planting @ 6l/ $m^2$. Thirty day old seedlings were planted at a spacing of 45 cm between plants and 60 cm between the rows with 24 plants in each plot.

Observations on wilt incidence at 30, 45, and 60 days after transplanting and yield /plot were recorded. Ten plants were randomly selected and labelled for taking observations on plant height, days to flowering and fruiting, number of fruits /plant, and yield / plant.

### 3.25.2.5. *Statistical analysis*

Analysis of variance was performed on the data collected from the experiment using statistical package, MSTAT (Freed, 1986). Multiple comparisons among the treatments were done using Duncan's Multiple Range Test (DMRT).

# Results

# 4. RESULTS

The present investigation was carried out to isolate efficient microbial degraders from lignin-tannin rich agrowastes and to develop a microbial consortium for the rapid degradation of these substrates. The efficacy of these composts were further evaluated against two major soil borne diseases of vegetables, bacterial wilt of tomato and leaf blight of amaranth under field condition.

## 4.1. STANDARDISATION OF DILUTION FACTORS FOR THE ISOLATION OF MICROBIAL DEGRADERS

Preliminary isolation of cellulose, lignin and tannin degraders was carried out using enriched as well as non enriched samples of soils and partially degraded ayurvedic wastes on selective media adopting standard protocols and the dilution factors for the isolation of various degraders were standardised. Dilutions of $10^{-7}$ and $10^{-5}$ which showed maximum countable colonies were selected for the isolation of cellulose degraders and $10^{-4}$ and $10^{-3}$ dilutions for both lignin and tannin degraders from enriched and non enriched samples respectively (Table 3).

## 4.2. ISOLATION AND ENUMERATION OF MICROBIAL DEGRADERS FROM COLLECTED SAMPLES

Microbial degraders were isolated from both enriched and non enriched 53 samples of soils and partially degraded substrates collected from various sources and locations. Quantitative estimation of degraders was carried out from these 106 samples using the dilutions standardised for each type of degrader and the data are presented in Tables 4, 5 and 6 (Plate 2).

Microbial population was higher in enriched samples as compared to non enriched ones and the count was higher in partially degraded substrates than the soil samples. Variation was observed in the case of tannin degraders, in which soil samples from teak plantation, mango orchards and coir pith dumping sites of Andiyoor (Pollachi) recorded maximum population. Among the samples, ayurvedic wastes recorded considerably high population of cellulose, lignin and

## Table 3. Standardisation of dilution factors for isolation of microbial degraders

| Dilutions | * Microbial population (No. of cfug-1) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cellulose degraders | | | | Lignin degraders | | | | Tannin degraders | | | |
| | Soil | | Substrate | | Soil | | Substrate | | Soil | | Substrate | |
| | Enriched | Non enriched | Enriched | Non enriched | Enriched | Non enriched | Enriched | Non enriched | Enriched | Non enriched | Enriched | Non enriched |
| $10^{-2}$ | Too numerous to count (TNTC) | TNTC | TNTC | TNTC | 109 | 95 | 142 | 103 | 118 | 102 | 131 | 89 |
| $10^{-3}$ | 165 | 143 | TNTC | TNTC | 68 | 58 | 71 | 59 | 67 | 52 | 69 | 61 |
| $10^{-4}$ | 110 | 84 | 128 | 103 | 54 | 35 | 48 | 24 | 56 | 38 | 41 | 27 |
| $10^{-5}$ | 61 | 42 | 103 | 57 | 29 | 19 | 11 | 7 | 27 | 15 | 4 | 2 |
| $10^{-6}$ | 43 | 28 | 89 | 37 | 13 | 8 | 8 | 3 | 5 | 2 | 0 | 0 |
| $10^{-7}$ | 24 | 16 | 54 | 11 | - | - | - | - | - | - | - | - |
| 10-8 | 5 | 1 | 36 | 6 | - | - | - | - | - | - | - | - |
| $10^{-9}$ | 0 | 0 | 11 | 2 | - | - | - | - | - | - | - | - |

* Mean of three replications

tannin degraders followed by the samples from forest areas. In addition, coir pith and leaf litters of teak and mango also recorded good population of lignin and tannin degraders.

### 4.2.1. Enumeration of cellulose degraders

Population of cellulose degraders showed wide variation among the substrates which ranged from 2 - 562 x $10^7$ $cfug^{-1}$ in enriched and 1- 380 x $10^5 cfug^{-1}$ in non enriched samples. Samples from ayurvedic units yielded highest population of 21- 562 x $10^7$ $cfug^{-1}$ and much difference was not observed among the locations. However, an increase in population was noticed in the *kashayam* waste as compared to *arishtam* samples collected from SD Pharmacy, Alappuzha and Kerala Ayurveda Ltd, Angamaly.

Samples from coir fibre extraction areas, forests and plantations also recorded considerable population of cellulose degraders. Of the three sources of coconut by products, population was found maximum in the samples from coir fibre extraction areas of Kalavoor (171 x $10^7$ $cfug^{-1}$) and Gopalapuram (142 x $10^7$ $cfug^{-1}$).

Substrates as well as soil samples from forest areas also recorded a population of 120 - 202 x $10^7$ $cfug^{-1}$. Like wise, leaf litter and soil samples from the areas of cashew, teak and mango yielded 124 -550 x $10^7$ $cfug^{-1}$ of cellulose degraders.

In addition, vermicompost, straw spent mushroom substrate dumping sites and termites were also found to be good sources of cellulose degraders, recording populations of 478 x $10^7$ $cfug^{-1}$, 244 x $10^7$ $cfug^{-1}$ and 39 x $10^7$ $cfug^{-1}$ respectively (Table 4).

### 4.2.2. Enumeration of lignin degraders

From the data shown in Table 5, it is observed that, the population of lignin degraders varied from 1- 684 x $10^4$ $cfug^{-1}$ and 1- 482 x $10^3$ $cfug^{-1}$ in enriched and non enriched samples respectively.

## Table 4. Enumeration of cellulose degraders from samples collected from different locations

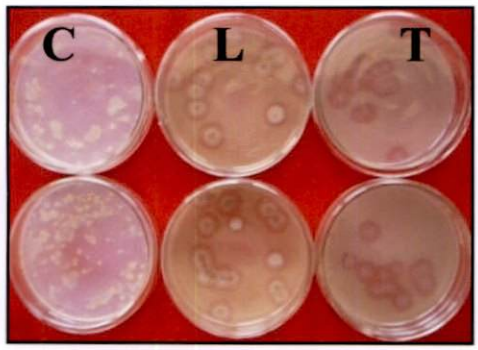
| Locations | *Microbial Population | | |
|---|---|---|---|
| | Sources | Enriched sample $1 \times 10^7$ cfug$^{-1}$ | Non enriched sample $1 \times 10^5$cfug$^{-1}$ |
| **Ayurvedic pharmaceutical units** | | | |
| 1.SD pharmacy | Kashayam waste | 225 | 93 |
| | Aristam waste | 59 | 56 |
| | Soil | 175 | 123 |
| 2. Kerala Ayurvedics Ltd. | Kashayam waste | 252 | 63 |
| | Aristam waste | 42 | 34 |
| | Soil | 21 | 20 |
| 3. Kottakkal Ayurvedics | Substrate | 233 | 83 |
| | Soil | 150 | 79 |
| 4. Oushadhi | Kashayam waste | 562 | 380 |
| 5. Vaidhyaratnum | Kashayam waste | 294 | 20 |
| **Coir retting area** | | | |
| 1. Thaneermukkam | Substrate | 15 | 24 |
| | Soil | 18 | 11 |
| **Coir fibre extraction areas** | | | |
| 1. Kalavoor | Substrate | 171 | 110 |
| | Soil | 48 | 37 |
| 2. Gopalapuram | Substrate | 142 | 113 |
| | Soil | 87 | 65 |
| 3. Unchavellampatty | substrate | 21 | 2 |
| **Coir pith dumping sites** | | | |
| 1. Andiyoor | Substrate | 39 | 36 |
| | Soil | 10 | 10 |
| 2. Adivaram | Substrate | 8 | 5 |
| | soil | 18 | 10 |
| 3. Kollarpatty | Substrate | 41 | 2 |
| | Soil | 37 | 4 |
| **Coconut byproducts mulched basins** | | | |
| 1. Ollukkara | Substrate | 14 | 10 |
| | Soil | 21 | 7 |
| 2. Thimmankuthu | Soil | 60 | 21 |
| **Elephant dung dumping sites** | | | |
| 1. Anakotta, Guruvayur | Substrate | 40 | 13 |
| | Soil | 10 | 11 |
| 2.Anaparambu, Thrissur | Substrate | 3 | 11 |
| | Soil | 6 | 5 |

| | | | |
|---|---|---|---|
| **Forest areas** | | | |
| 1. Dhoni | Substrate | 202 | 57 |
| | Soil | 31 | 17 |
| 2. Poonjar | Soil | 18 | 10 |
| 3. Silent valley | Soil | 120 | 5 |
| 4. Chimmony | Soil | 180 | 1 |
| **Cashew plantation** | | | |
| 1.Madakkathara | Substrate | 180 | 120 |
| | Soil | 48 | 4 |
| **Teak plantations** | | | |
| | Substrate | 124 | 98 |
| 1.Peechi | Soil | 550 | 144 |
| 2.Nilambur | Substrate | 6 | 2 |
| | Soil | 4 | 3 |
| **Mango orchards** | | | |
| 1.Vellanikkara | Substrate | 12 | 2 |
| | soil | 5 | 1 |
| 2.Ollukkara | Substrate | 96 | 57 |
| | Soil | 160 | 17 |
| 3. Ozhalapathy | Substrate | 156 | 71 |
| | Soil | 95 | 54 |
| **Compost pit** Vellanikkara | Soil | 48 | 34 |
| **Vermi compost** Vellanikkara | Substrate | 478 | 340 |
| **Spent mushroom substrate dumping sites** | | | |
| 1.Straw | Substrate | 244 | 97 |
| Vellanikkara | Soil | 27 | 15 |
| | | | |
| 2. Saw dust | Substrate | 12 | 1 |
| Vellankkara | Soil | 2 | 8 |
| **Saw mill** Paravattani | Substrate | 7 | 5 |
| | Soil | 4 | 3 |
| **Termite mound** Vellanikkara | | 39 | 21 |
| **Fresh Cow dung** Vellanikkara | | 12 | 6 |

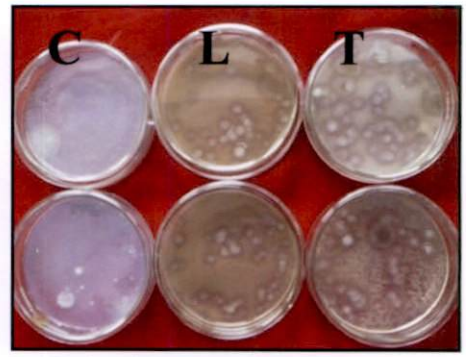
*Mean of three replications

# PLATE 2

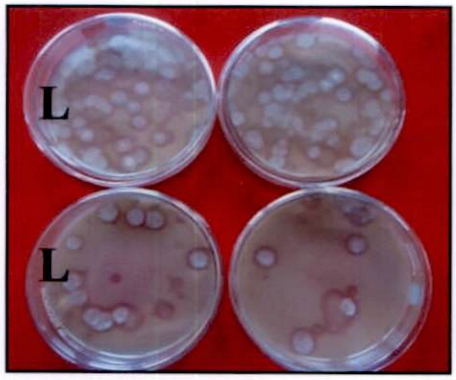
## ISOLATION OF MICROBIAL DEGRADERS



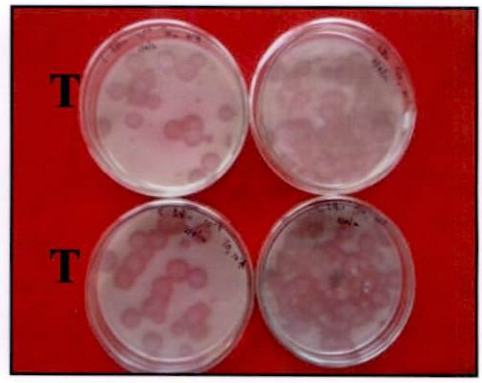

Ayurvedic pharmaceutical unit




Coir pith dumping site

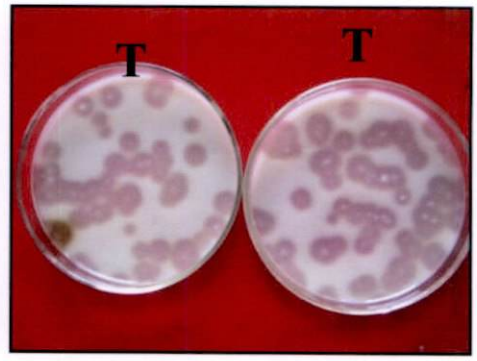



Coir fibre extraction area

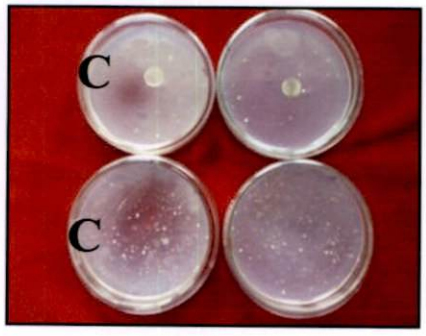



Teak plantation


Mango orchard




Forest area




SMS straw


Saw mill

C – Cellulose, L-Lignin , T-Tannin

## Table 5. Enumeration of lignin degraders from samples collected from different locations

| Locations | *Microbial population | | |
|---|---|---|---|
| | Sources | Enriched sample $1 \times 10^4$ cfug$^{-1}$ | Non enriched sample $1 \times 10^3$ cfug$^{-1}$ |
| **Ayurvedic pharmaceutical units** | | | |
| 1.SD pharmacy | Kashayam waste | 158 | 43 |
| | Aristam waste | 123 | 116 |
| | Soil | 27 | 23 |
| 2. Kerala ayurvedics Ltd. | Kashayam waste | 162 | 56 |
| | Aristam waste | 76 | 68 |
| | Soil | 24 | 11 |
| 3. Kottakkal ayurvedics | Substrate | 54 | 37 |
| | Soil | 24 | 24 |
| 4. Oushadhi | Kashayam waste | 684 | 482 |
| 5. Vaidhyaratnum | Kashayam waste | 15 | 3 |
| **Coir retting area** | | | |
| 1. Thaneermukkam | Substrate | 30 | 12 |
| | Soil | 35 | 25 |
| **Coir fibre extraction areas** | | | |
| 1. Kalavoor, Alappuzha | Substrate | 42 | 23 |
| | Soil | 15 | 9 |
| 2. Gopalapuram, | Substrate | 38 | 52 |
| | Soil | 24 | 19 |
| 3. Unchavellampatty | substrate | 34 | 10 |
| **Coir pith dumping sites** | | | |
| 1. Andiyoor | Substrate | 244 | 18 |
| | Soil | 116 | 8 |
| 2.Adivaram | Substrate | 93 | 76 |
| | soil | 38 | 3 |
| 3. Kollarpatty | Substrate | 18 | 5 |
| | Soil | 12 | 3 |
| **Coconut byproducts mulched basins** | | | |
| 1.Ollukkara | Substrate | 11 | 17 |
| | Soil | 17 | 17 |
| 2. Thimmankuthu | Soil | 27 | 11 |
| **Elephant dung dumping sites** | | | |
| 1. Anakotta, Guruvayur | Substrate | 56 | 56 |
| | Soil | 48 | 13 |
| 2.Anaparambu, Thrissur | Substrate | 41 | 5 |
| | Soil | 41 | 14 |

| | | | |
|---|---|---|---|
| **Forest areas** | | | |
| 1.Dhoni | Substrate | 223 | 43 |
| | Soil | 142 | 36 |
| 2. Poonjar | Soil | 50 | 13 |
| 3. Silent valley | Soil | 80 | 4 |
| 4. Chimmony | Soil | 140 | 8 |
| **Cashew plantation** | | | |
| 1.Madakkathara | Substrate | 30 | 19 |
| | Soil | 50 | 22 |
| **Teak plantations** | | | |
| 1.Peechi | Substrate | 276 | 85 |
| | Soil | 136 | 83 |
| 2.Nilambur | Substrate | 8 | 1 |
| | Soil | 5 | 2 |
| **Mango orchards** | | | |
| 1.Vellanikkara | Substrate | 42 | 43 |
| | Soil | 25 | 20 |
| 2.Ollukkara | Substrate | 24 | 12 |
| | Soil | 244 | 37 |
| 3. Ozhalapathy | Substrate | 174 | 83 |
| | Soil | 135 | 61 |
| **Compost pit** Vellanikkara | Soil | 57 | 46 |
| **Vermi compost** Vellanikkara | Substrate | 113 | 16 |
| **Spent mushroom substrate dumping sites** | | | |
| 1.Straw Vellanikkara | Substrate | 7 | 3 |
| | Soil | 16 | 9 |
| 2. Saw dust | Substrate | 36 | 25 |
| Vellanikkara | Soil | 12 | 1 |
| **Saw mill** Paravattani | Substrate | 11 | 7 |
| | Soil | 51 | 43 |
| **Termite mound** Vellanikkara | | 0 | 0 |
| **Fresh Cow dung** Vellanikkara | | 6 | 4 |

* Mean of three replications

Like cellulose degraders, samples from ayurvedic pharmaceutical units, forest areas, teak plantations and mango orchards recorded high population of lignin degraders, of which, ayurvedic wastes recorded the maximum ($684 \times 10^4$ $cfug^{-1}$).

Of the three sources of coconut by products, samples from coir pith dumping site of Andiyoor, recorded maximum count ($244 \times 10^4$ $cfug^{-1}$). Substrates and soil samples from Dhoni forest, Peechi teak plantation and mango orchards also recorded high population of 142 - 223 x $10^4 cfug^{-1}$, 136 - 276 x $10^4$ $cfug^{-1}$ and 135 – 244 x $10^4$ $cfug^{-1}$ respectively.

It was also noted that, vermicompost, elephant dung dumping sites and saw mills were also found to be good sources of lignin degraders.

### 4.2.3. Enumeration of tannin degraders

Data furnished in Table 6 showed that, population of tannin degraders varied from 5 - 680 x $10^4$ $cfug^{-1}$ in enriched and 1- 223 x $10^4$ $cfug^{-1}$ in non enriched samples. Even though, the samples from ayurvedic units yielded good population of tannin degraders, the highest count was recorded in the samples from teak plantations (232 - 680 x $10^4$ $cfug^{-1}$) followed by mango orchards (104 - 340 x $10^4$ $cfug^{-1}$). Among the samples from various ayurvedic units, samples from SD pharmacy recorded maximum population (104 -180 x $10^4$ $cfug^{-1}$).

In addition, samples from coir pith dumping sites of Andiyoor, Dhoni forest and vermicompost also recorded population of 120 x $10^4$, 196 x $10^4$ and 138 x $10^4$ $cfug^{-1}$ respectively.

From the enumerated microbial population, 378 organisms were selected based on diffusion test, comprising of 125 cellulose,133 lignin and 120 tannin degraders. Cellulose degraders recorded all the three types of organisms with dominance of actinomycetes (58) followed by fungi (40) and bacteria (27). In case of lignin and tannin degraders, fungi were the predominant type. There were 127 fungi, five bacteria and one actinomycete in lignin and 119 fungi and one yeast in

## Table 6. Enumeration of tannin degraders from collected samples from different locations

| Locations | *Microbial population | | |
|---|---|---|---|
| | Sources | Enriched sample $1 \times 10^4$ cfug$^{-1}$ | Non enriched sample $1 \times 10^3$ cfug$^{-1}$ |
| **Ayurvedic pharmaceutical units** | | | |
| 1.SD pharmacy | Kashayam waste | 145 | 40 |
| | Aristam waste | 140 | 138 |
| | Soil | 180 | 50 |
| 2. Kerala ayurvedics Ltd. | Kashayam waste | 39 | 9 |
| | Aristam waste | 58 | 46 |
| | Soil | 37 | 29 |
| 3. Kottakkal ayurvedics | Substrate | 59 | 45 |
| | Soil | 17 | 9 |
| 4. Oushadhi | Kashayam waste | 84 | 65 |
| 5. Vaidhyaratnum | Kashayam waste | 14 | 2 |
| **Coir retting area** | | | |
| 1. Thaneermukkam | Substrate | 33 | 23 |
| | Soil | 32 | 19 |
| **Coir fibre extraction areas** | | | |
| 1. Kalavoor | Substrate | 33 | 34 |
| | Soil | 53 | 35 |
| 2. Gopalapuram | Substrate | 23 | 27 |
| | Soil | 18 | 21 |
| 3. Unchavellampatty | substrate | 16 | 11 |
| **Coir pith dumping sites** | | | |
| 1. Andiyoor | Substrate | 13 | 14 |
| | Soil | 120 | 7 |
| 2. Adivaram | Substrate | 81 | 63 |
| | Soil | 33 | 10 |
| 3. Kollarpatty | Substrate | 14 | 4 |
| | Soil | 9 | 2 |
| **Coconut byproducts mulched basins** | | | |
| 1. Ollukkara | Substrate | 34 | 8 |
| | Soil | 19 | 9 |
| 2. Thimmankuthu | Soil | 16 | 7 |
| **Elephant dung dumping sites** | | | |
| 1. Anakotta, Guruvayur | Substrate | 28 | 29 |
| | Soil | 29 | 6 |
| 2.Anaparambu, Thrissur | Substrate | 17 | 6 |
| | Soil | 30 | 15 |

| | | | |
|---|---|---|---|
| **Forest areas** | | | |
| 1. Dhoni | Substrate | 196 | 29 |
| | Soil | 18 | 22 |
| 2. Poonjar | Soil | 16 | 10 |
| 3. Silent valley | Soil | 13 | 3 |
| 4. Chimmony | Soil | 12 | 0 |
| **Cashew plantation** | | | |
| 1.Madakkathara | Substrate | 28 | 11 |
| | Soil | 40 | 21 |
| **Teak plantations** | | | |
| | Substrate | 232 | 104 |
| 1.Peechi | Soil | 680 | 223 |
| 2.Nilambur | Substrate | 5 | 4 |
| | Soil | 5 | 1 |
| **Mango orchards** | | | |
| 1.Vellanikkara | Substrate | 20 | 38 |
| | Soil | 41 | 30 |
| 2.Ollukkara | Substrate | 44 | 32 |
| | Soil | 340 | 26 |
| 3. Ozhalapathy | Substrate | 92 | 76 |
| | Soil | 104 | 69 |
| **Compost pit** Vellanikkara | Soil | 26 | 20 |
| **Vermi compost** Vellanikkara | Substrate | 138 | 12 |
| **Spent mushroom substrate dumping sites** | | | |
| 1.Straw Vellanikkara | Substrate | 7 | 1 |
| | Soil | 10 | 4 |
| 2. Saw dust | Substrate | 4 | 2 |
| Vellanikkara | Soil | 82 | 68 |
| **Saw mill** Paravattani | Substrate | 5 | 3 |
| | Soil | 60 | 34 |
| **Termite mound** Vellanikkara | | 0 | 0 |
| **Fresh Cow dung** Vellanikkara | | 24 | 18 |

* Mean of three replications

tannin degraders. Maximum types of organisms were observed in the samples collected from ayurvedic units (Table 7).

## 4.3. *IN VITRO* EVALUATION OF MICROBIAL DEGRADERS

Three hundred and seventy eight degraders selected from the previous study were further tested individually on respective selective medium for their potential to utilize respective chemical component. The degrading ability was assessed using 1- 5 scale score chart based on the size of diffusion zones produced. The colony characters of organisms were also recorded and the results are summarised in Tables 8, 9 and 10.

In *in vitro* screening, the size of diffusion zones varied with different degraders. The diffusion zones produced by cellulose degraders varied from 1.2 - 6.6 cm and for lignin and tannin degraders, 1.1 -5.1 cm and 1.8 – 5.5 cm respectively (Table 11).

### 4.3.1. *In vitro* evaluation of cellulose degraders

Of the 125 cellulose degraders tested *in vitro*, 31 organisms showed the maximum score of 5, recording > 4.0 cm diffusion zones followed by 22 with score 4 and 39 with score 3 and others were least effective. Maximum diffusion zones were observed with two fungal degraders, ACF- 4 (6.6 cm) from ayurvedic waste and TCF-5 (6.0 cm) from teak leaves which belonged to *Aspergillus* spp. In addition, a *Trichoderma* isolate (ECF-2) from elephant dung also showed good diffusion zone of 5.5 cm (Plate 3 A).

Among the bacteria and actinomycetes, CdCB-1 from fresh cow dung, CCB -8 from coconut by products and CaCA-3 (actinomycete) from cashew litters were the most effective ones and recorded maximum diffusion zones of 5.7 cm, 5.5 cm and 4.2 cm respectively (Table 8).

It was also observed that, among the different samples, cellulose degraders isolated from ayurvedic units, leaf litters, forest areas, coconut by products mulched basins and coir fibre extraction areas were the efficient ones recording

## Table 7. Types of microbial degraders from various samples

| Sl no. | Source | Cellulose | | | | Lignin | | | | Tannin | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F | B | A | Total | F | B | A | Total | F | B | A | Total |
| 1. | Ayurvedic units -5 | 15 | 5 | 18 | 38 | 30 | 2 | 1 | 33 | 25 | 1 | 0 | 26 |
| 2. | Elephant dung dumping sites -2 | 1 | 2 | 8 | 11 | 13 | 0 | 0 | 13 | 12 | 0 | 0 | 12 |
| 3. | Coir retting area-1 | 0 | 2 | 0 | 2 | 3 | 0 | 0 | 3 | 3 | 0 | 0 | 3 |
| 4. | Coir fibre extraction areas - 3 | 3 | 3 | 4 | 10 | 11 | 0 | 0 | 11 | 8 | 0 | 0 | 8 |
| 5. | Coir pith dumping sites-3 | 3 | 2 | 7 | 12 | 14 | 0 | 0 | 14 | 14 | 0 | 0 | 14 |
| 6. | Coconut by product mulched sites -2 | 0 | 4 | 1 | 5 | 8 | 0 | 0 | 8 | 8 | 0 | 0 | 8 |
| 7. | Forest areas -4 | 1 | 3 | 0 | 4 | 8 | 0 | 0 | 8 | 10 | 0 | 0 | 10 |
| 8. | Cashew plantation-1 | 3 | 1 | 6 | 10 | 10 | 0 | 0 | 10 | 9 | 0 | 0 | 9 |
| 9. | Teak plantations -2 | 5 | 0 | 2 | 7 | 8 | 0 | 0 | 8 | 8 | 0 | 0 | 8 |
| 10. | Mango orchard -3 | 4 | 0 | 0 | 4 | 7 | 0 | 0 | 7 | 10 | 0 | 0 | 10 |
| 11. | Compost pits -2 | 0 | 0 | 7 | 7 | 7 | 0 | 0 | 7 | 3 | 0 | 0 | 3 |
| 12. | Spent mushroom substrate (SMS) dumping sites -2 | 3 | 1 | 2 | 6 | 4 | 0 | 0 | 4 | 5 | 0 | 0 | 5 |
| 13. | Saw mill | 0 | 1 | 0 | 1 | 3 | 3 | 0 | 6 | 2 | 0 | 0 | 2 |
| 14. | Termite mound | 2 | 1 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | Fresh cow dung | 0 | 2 | 1 | 3 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 2 |
| | **Total** | **40** | **27** | **58** | **125** | **127** | **5** | **1** | **133** | **119** | **1** | **0** | **120** |

F- Fungi, B- Bacteria, A- Actinomycete

## Table 8. *In vitro* evaluation of cellulose degraders

| Sl.No. | Isolate No. | * Diameter of diffusion zone (cm) | Score | Colony characters |
|---|---|---|---|---|
| AYURVEDIC PHARMACEUTICALS UNITS | | | | |
| FUNGI | | | | |
| 1. | ACF-1 | 1.1 | 2 | Sparse white mycelium, dark green sporulation in concentric pattern (*Aspergillus* sp.) |
| 2. | ACF-2 | 4.6 | 5 | White mycelium, thick olive green spores, dark orange metabolite in the media (*A. flavus*) |
| 3. | ACF-3 | 2.4 | 3 | Light pink colony later turning grey in colour, reverse side dark pink |
| 4. | ACF-4 | 6.6 | 5 | Sparse white mycelium with olive green sporulation (*A. flavus*) |
| 5. | ACF-5 | 5.0 | 5 | White mycelium, thick black sporulation (*A. niger*) |
| 6. | ACF-6 | 4.8 | 5 | Dark brown mycelium, slow growth in concentric pattern (*Aspergillus* sp.) |
| 7. | ACF-7 | 3.7 | 4 | White mycelium with black sporulation in concentric pattern (*Aspergillus* sp.) |
| 8. | ACF-8 | 3.5 | 4 | Dark brown mycelium, slow growth in concentric pattern |
| 9. | ACF-9 | 3.2 | 4 | White mycelium,dark green, sporulation, fast growth |
| 10. | ACF-10 | 5.5 | 5 | White mycelium, black sporulation (*A. niger*) |
| 11. | ACF-11 | 5.8 | 5 | White fluffy rough textured mycelium, no visible sporulation (Mushroom fungus growth) |
| 12. | ACF-12 | 3.8 | 4 | White mycelium, greyish powdery sporulation in concentric pattern |
| 13. | ACF-13 | 5.5 | 5 | White mycelium, olive green sporulation (*A. flavus.*) |
| 14. | ACF-14 | 5.0 | 5 | White fluffy mycelium. No sporulation (mushroom fungus growth) |

| | | | | |
|---|---|---|---|---|
| 15. | ACF-15 | 4.4 | 5 | White mycelium, alternate yellow and green sporulation (*Aspergillus* sp.) |
| | | **COIR PITH** | | |
| 16. | CCF-1 | 2.7 | 3 | Black raised growth, very small isolated colonies, slow growth. |
| 17. | CCF-2 | 3.1 | 4 | White mycelium, green sporulation |
| 18. | CCF-3 | 1.0 | 1 | Dark green spore mass on sparse mycelium. |
| 19. | CCF-4 | 2.7 | 3 | Thick white mycelium, hyaline spores (*Fusarium* sp.) |
| 20. | CCF-5 | 3.1 | 4 | Brown sporulation, yellow metabolite in the media |
| 21. | CCF-6 | 2.0 | 3 | Dark green sporulation, olive green colour on the reverse side (*Aspergillus* sp.) |
| | | **ELEPHANT DUNG** | | |
| 22. | ECF-2 | 5.5 | 5 | White mycelium, light green sporulation (*Trichoderma* sp.) |
| | | **FOREST AREA** | | |
| 23. | FCF-1 | 5.6 | 5 | Sparse white mycelium, olive green spores in concentric pattern (*A. flavus*) |
| | | **CASHEW PLANTATION** | | |
| 24. | CaCF-1 | 5.4 | 5 | White mycelium, dark green sporulation at the centre (*A. flavus*) |
| 25. | CaCF-2 | 1.0 | 2 | Greyish green powdery sporulation. No visible mycelial growth. |
| 26. | CaCF-3 | 1.3 | 2 | Yellowish white concentric growth, pink sporulation |
| | | **TEAK PLANTATION** | | |
| 27. | TCF-1 | 3.5 | 4 | White mycelium, black sporulation |
| 28. | TCF-2 | 4.7 | 5 | White mycelial growth, olive green sporulation, yellow metabolite in the media (*A. flavus*) |
| 29. | TCF-3 | 5.5 | 5 | Yellowish green mycelial growth, olive green sporulation (*A. flavus*) |

| | | | | |
|---|---|---|---|---|
| 30. | TCF-4 | 4.5 | 5 | White mycelium, olive green sporulation in concentric pattern (*A. flavus*) |
| 31. | TCF-5 | 6.0 | 5 | Green sporulation in concentric pattern (*Aspergillus* sp.) |
| | | **MANGO ORCHARD** | | |
| 32. | MCF-1 | 4.0 | 5 | White mycelium, black sporulation (*A. niger*) |
| 33. | MCF-2 | 4.5 | 5 | White fluffy mycelium, no visible sporulation, very slow growth (mushroom fungus growth). |
| 34. | MCF-3 | 3.9 | 4 | White mycelium, turning lemon yellow in colour, dark green sporulation at the centre. |
| 35. | MCF-4 | 5.6 | 5 | White mycelium, black spores in concentric pattern, fast growth (*A. niger*) |
| | | **SPENT MUSHROOM SUBSTRATE** | | |
| 36. | SMCF-1 | 4.6 | 5 | White mycelium, olive green sporulation, orange yellow metabolite (*A. flavus.*) |
| 37. | SMCF-2 | 6.1 | 5 | White mycelium, olive green spores, isolated colonies. (*A . flavus*) |
| 38. | SMCF-3 | 3.3 | 4 | Lemon yellow mycelium later, centre turns pink and concentric growth. |
| | | **TERMITE MOUND** | | |
| 39 | TmCF-1 | 3.3 | 4 | Black fluffy mycelium, small isolated colonies. |
| 40 | TmCF-2 | 1.8 | 2 | Dark green scattered spore masses. |
| | | **ACTINOMYCTES** | | |
| | | **AYURVEDIC PHARMACEUTICAL UNITS** | | |
| 41. | ACA-1 | 2.0 | 3 | White powdery growth on dried creamy base |
| 42. | ACA-2 | 2.5 | 3 | White powdery sporulation |
| 43. | ACA-3 | 3.2 | 4 | Pinkish white crusty appearance, white dried wavy margins |

| | | | | |
|---|---|---|---|---|
| 44. | ACA-4 | 3.4 | 4 | Greyish powdery growth, thin white margins, yellow metabolite in the media. |
| 45. | ACA-5 | 3.7 | 4 | Dark grey powdery growth with yellow on the reverse side, white margin. |
| 46. | ACA-6 | 3.2 | 4 | Light grey powdery growth, black colour on the reverse side of the plate |
| 47. | ACA-7 | 2.3 | 3 | Milky white powdery growth, wavy margins. |
| 48. | ACA-8 | 3.5 | 4 | Greyish white raised powdery growth, powdery pinkish colour on the reverse side. |
| 49. | ACA-9 | 2.3 | 3 | Grey powdery growth, reverse side of the colony cream colour with pinkish centre. |
| 50 | ACA-10 | 1.2 | 2 | Greyish powdery growth |
| 51. | ACA-11 | 2.9 | 3 | Very thin, grey, powdery growth, creamy white colour on the reverse side |
| 52. | ACA-12 | 2.4 | 3 | Bluish grey powdery growth, white margins, bluish colour with yellow margins on the reverse side |
| 53. | ACA-13 | 2.0 | 3 | Bluish grey colour on the margins ,greyish centre, reverse side black in colour |
| 54. | ACA-14 | 1.2 | 2 | Bluish grey powdery growth with white margins. |
| 55. | ACA-15 | 2.0 | 3 | Creamy white slimy growth later reddish powdery growth at the centre. |
| 56. | ACA-16 | 2.2 | 3 | Brownish white powdery growth, black colour in the media, reverse side brown in colour |
| 57. | ACA-17 | 2.3 | 3 | Concentric growth pattern of colony, white powdery growth, dark pink colour on reverse side |
| 58. | ACA-18 | 1.3 | 2 | Pinkish powdery growth, and reverse side pink in colour |
| **COIR PITH** | | | | |
| 59 | CCA-1 | 2.5 | 3 | Dark grey powdery growth, yellow metabolite in media and yellow colour on the reverse side |

| | | | | |
|---|---|---|---|---|
| 60 | CCA-2 | 2.7 | 3 | Dark grey powdery growth with white margins, creamy yellow colour on the reverse side |
| 61 | CCA-3 | 1.7 | 2 | Greyish white powdery growth, round edged colonies, light yellow colour on the reverse side. |
| 62 | CCA-4 | 2.5 | 3 | Pink, greyish growth, pink colour on the reverse side. |
| 63 | CCA-5 | 2.7 | 3 | White powdery growth. |
| 64 | CCA-6 | 2.8 | 3 | White powdery growth, brown colour on the |
| 65 | CCA-7 | 2.3 | 3 | White powdery growth. |
| 66 | CCA-8 | 2.1 | 3 | White powdery growth. |
| 67 | CCA-9 | 2.6 | 3 | Pinkish white powdery growth, pink colour on the reverse side. |
| 68 | CCA-10 | 3.0 | 4 | Creamy white powdery growth. |
| 69 | CCA-11 | 3.1 | 5 | Green powdery growth. |
| 70 | CCA-12 | 3.0 | 4 | Greyish powdery growth. |
| **ELEPHANT DUNG** | | | | |
| 71 | ECA-1 | 1.6 | 2 | Greyish powdery growth, black colour on the reverse side. |
| 72 | ECA-2 | 1.5 | 2 | Greyish powdery growth. |
| 73 | ECA-3 | 1.9 | 2 | Yellowish orange, powdery growth, light orange colour on the reverse side of the plate. |
| 74 | ECA-4 | 1.5 | 2 | Pure white, powdery, growth. |
| 75 | ECA-5 | 1.7 | 2 | Greyish white, powdery growth, yellow colour on the reverse side of the plate. |
| 76 | ECA-6 | 2.3 | 3 | Cream coloured powdery growth, light yellow colour on the reverse side. |
| 77 | ECA-7 | 1.8 | 2 | Light orange, powdery growth, dark orange colour on the reverse side of the plate. |
| 78 | ECA-8 | 2.9 | 3 | Whitish grey coloured, powdery growth, brownish yellow metabolite on the reverse side. |
| **CASHEW PLANTATION** | | | | |
| 79 | CaCA-1 | 3.1 | 5 | White powdery growth |
| 80 | CaCA-2 | 2.3 | 3 | White powdery rounded growth on the streak, yellow colour on reverse side |

| | | | | |
|---|---|---|---|---|
| 81 | CaCA-3 | 4.2 | 5 | Pinkish white powdery growth, pink colour on reverse side. |
| 82 | CaCA-4 | 3.2 | 4 | White powdery growth, spreading wavy margins. |
| 83 | CaCA-5 | 3.3 | 4 | Bluish powdery growth, with round edges – different species |
| 84 | CaCA-6 | 2.3 | 3 | Blackish grey powdery growth, round edges,white margins. |
| **TEAK PLANTATION** | | | | |
| 85 | TCA-1 | 2.6 | 3 | Yellow powdery growth. |
| 86 | TCA-2 | 2.1 | 3 | White slimy powdery growth |
| **COMPOST PIT** | | | | |
| 87 | CpCA-1 | 2.8 | 3 | Thick white powdery growth, reverse side light pink in colour |
| 88 | CpCA-2 | 1.4 | 2 | Light grey powdery growth with watery exudate . |
| 89 | CpCA-3 | 3.0 | 4 | Pure white fluffy thick powdery growth, round growth on the streak, pink colour on the reverse side. |
| 90 | CpCA-4 | 2.3 | 3 | Bluish grey powdery colony. Black colour at the centre on the back side. |
| **VERMICOMPOST** | | | | |
| 91 | VeCA-1 | 1.8 | 2 | Light pink powdery growth. Lower surface white in colour |
| 92 | VeCA-2 | 3.2 | 4 | Grey powdery growth |
| 93 | VeCA-3 | 2.2 | 3 | Light pink powdery growth |
| **SPENT MUSHROOM SUBSTRATE** | | | | |
| 94 | SMCA-1 | 2.0 | 2 | Creamy white powdery growth. |
| 95 | SMCA-2 | 1.8 | 3 | White mycelial growth |
| **TERMITE MOUND** | | | | |
| 96 | TmCA-1 | 2.8 | 3 | White mycelium |
| 97 | TmCA-2 | 3.3 | 4 | White powdery growth, discontinuous growth, round edges for streak, black colour on reverse side of the plate. |
| **COW DUNG** | | | | |
| 98 | CdCA -1 | 1.6 | 2 | Thick white powdery growth. |
| **BACTERIA** | | | | |
| **AYURVEDIC PHARMACEUTICAL UNITS** | | | | |
| 99 | ACB-1 | 1.1 | 2 | white , slimy, fast growing |
| 100 | ACB-2 | 5.5 | 5 | Yellow, slimy, fast growing |

| | | | | |
|---|---|---|---|---|
| 101 | ACB-3 | 5.3 | 5 | white ,slimy, fast growing |
| 102 | ACB-4 | 4.2 | 5 | White, slimy, fast growing. |
| 103 | ACB-5 | 3.5 | 4 | Yellow, slimy, slow growing |
| **COIR PITH** | | | | |
| 104 | CCB-1 | 3.3 | 4 | Yellow, fluidal, fast growing |
| 105 | CCB-2 | 3.3 | 4 | White, slimy with serrated margins |
| 106 | CCB-3 | 4.8 | 5 | White, with fluorescence, fast growing |
| 107 | CCB-4 | 4.3 | 5 | Yellow, fluidal |
| 108 | CCB-5 | 2.0 | 3 | White, dry colony |
| 109 | CCB-6 | 2.3 | 3 | white ,slimy |
| 110 | CCB-7 | 2.0 | 3 | Cream , slimy |
| 111 | CCB-8 | 5.5 | 5 | Deep yellow, dry |
| 112 | CCB-9 | 4.8 | 5 | Yellowish fluidal colony with white centre |
| 113 | CCB-10 | 2.4 | 3 | White, fluidal |
| 114 | CCB-11 | 2.5 | 3 | Cream,slimy |
| **ELEPHANT DUNG** | | | | |
| 115 | ECB-3 | 3.0 | 4 | Yellow , fast growing |
| 116 | ECB-4 | 3.1 | 4 | White, slimy , fast growing |
| **FOREST AREA** | | | | |
| 117 | FCB-1 | 2.7 | 3 | Dark yellow dry |
| 118 | FCB-2 | 3.1 | 4 | Violet, slow growing |
| 119 | FCB-3 | 4.8 | 5 | Yellow, fast growing |
| **CASHEW PLANTATION** | | | | |
| 120 | CaCB-1 | 1.2 | 2 | White, slimy fast growing |
| **SPENT MUSHROOM SUBSTRATE** | | | | |
| 121 | SMCB-1 | 1.0 | 2 | White, slimy |
| **SAW MILL** | | | | |
| 122 | SCB-1 | 1.6 | 2 | White, slimy |
| **TERMITE MOUND** | | | | |
| 123 | TmCB-1 | 3.3 | 4 | Milky white, slimy, fluidal |
| **FRESH COW DUNG** | | | | |
| 124 | CdCB-1 | 5.7 | 5 | Deep yellow, slimy, fast growing |
| 125 | CdCB-2 | 4.8 | 5 | Deep yellow slimy, fast growing |

*Mean of two replications

**1-5 Scale score chart**

| Score | Diameter of diffusion zone (cm) |
|---|---|
| 1 | < 1 |
| 2 | 1- 2 |
| 3 | 2- 3 |
| 4 | 3 -4 |
| 5 | > 4 |

diffusion zones ranged from 4.5 - 6.6 cm. In addition, termite mound and fresh cow dung also yielded efficient degraders with zone range of 4.0 - 5.7 cm (Table 11).

### 4.3.2. *In vitro* evaluation of lignin degraders

One hundred and thirty three lignin degraders were evaluated individually on the selective medium for the lignin degrading potentiality and results are given in Table 9.

It is observed from the table that, 30 per cent of degraders showed maximum score of 5 followed by 36 per cent and 23 per cent with scores 4 and 3. Maximum diffusion zones were recorded by fungal degraders from coir pith, CLF-33 (5.1 cm) and CLF-14 (5.0 cm), belonged to *Aspergillus* spp. Fungal degraders other than *Aspergillus* spp. *viz.* ALF-26, CaLF-5, TLF-8 and CLF-13 were also efficient and recorded diffusion zones > 3 cm and the actinomycete, ALA-1 showed a zone of 2.8 cm and these were selected for further studies (Plate 3 B).

It is noticed from Table 11 that, among the various samples, degraders isolated from coconut by products were the most effective, followed by the degraders from ayurvedic wastes, leaf litters, forest areas, elephant dung, composts and saw mill recording diffusion zones ranged from 4.1 -5.1 cm.

### 4.3.3. *In vitro* evaluation of tannin degraders

Of the 120 tannin degraders screened, 28 per cent recorded score - 5, 47 per cent score - 4 and 24 per cent with score - 3. Among the degraders, maximum diffusion zones were recorded by CTF-25 (5.5 cm) and CTF -33 (5.2 cm), the fungal degraders from coir pith, which belonged to *Aspergillus* spp. (Table 10) (Plate 3 C).

It is noted from Table 11 that, among the various samples, degraders from coconut by products were the most effective, followed by ayurvedic waste, elephant dung, leaf litters, and forest areas, with diffusion zones ranged from

## Table 11. Consolidated data on *in vitro* performance of various degraders

| Sl.No. | Source | Diffusion zone range (cm) | | |
|---|---|---|---|---|
| | | Cellulose degrader | Lignin degrader | Tannin degrader |
| 1 | Ayurvedic waste | 1.2 - 6.6 | 2.0 - 4.7 | 2.4 - 4.8 |
| 2 | Elephant dung dumping sites | 1.2 - 3.1 | 1.7 - 4.1 | 1.8 - 4.6 |
| 3 | Coir retting area | 3.3 - 4.3 | 3.0 - 3.9 | 3.5 – 4.0 |
| 4 | Coir fibre extraction areas | 1.7 - 4.5 | 2.8 - 4.5 | 1.8 - 4.8 |
| 5 | Coir pith dumping sites | 1.8 - 2.9 | 2.0 – 5.1 | 3.1 – 5.5 |
| 6 | Coconut by products mulching sites | 2.4 - 5.5 | 1.8 – 5.1 | 1.9 – 5.2 |
| 7 | Teak plantations | 2.1 - 6.0 | 3.1 - 4.1 | 3.0 - 4.6 |
| 8 | Mango Orchards | 4.3 - 4.5 | 2.0 - 4.6 | 2.5 - 4.8 |
| 9 | Cashew plantation | 2.3 - 5.4 | 2.7 - 4.2 | 2.7 - 4.0 |
| 10 | Compost pits | 1.4 - 3.2 | 2.8 – 4.5 | 3.6 - 4.0 |
| 11 | Termite mound | 2.8 - 4.0 | - | - |
| 12 | Spent mushroom substrate dumping sites | 1.8 – 3.5 | 2.9 – 3.9 | 3.2 – 4.0 |
| 13 | Saw mill | 1.6 | 1.1 – 4.1 | 2.5 – 3.9 |
| 14 | Forest areas | 2.6 – 5.9 | 2.8 – 4.7 | 2.3 - 4.9 |
| 15 | Fresh cow dung | 4.8 – 5.7 | 2.1 - 3.9 | 2.4 – 4.0 |
| | **Range of diffusion zone** | **1.2 - 6.6** | **1.1 – 5.1** | **1.8 – 5.5** |

## Table 9. *In vitro* evaluation of lignin degraders

| Sl.No. | Isolate No. | * Diameter of diffusion zone (cm) | Score | Colony characters |
|---|---|---|---|---|
| FUNGI | | | | |
| AYURVEDIC PHARMACEUTICAL UNITS | | | | |
| 1. | ALF-1 | 4.0 | 5 | Sparse white mycelium with black sporulation at the centre (*Aspergillus* sp.) |
| 2. | ALF-2 | 4.0 | 5 | White fluffy mycelium with olive green sporulation (*A. flavus*) |
| 3. | ALF-3 | 4.0 | 5 | White fluffy, sparse, mycelium, black spores in concentric pattern fast growth (*A.niger*) |
| 4. | ALF-4 | 3.4 | 4 | White, sparse mycelium olive green spores, fast growth and sporulation (*A. flavus*) |
| 5. | ALF-5 | 2.7 | 3 | Sparse white mycelium, black sporulation, fast growth(*Aspergillus* sp.). |
| 6. | ALF-6 | 2.3 | 3 | White, raised mycelium, fast growth, black sporulation at the centre (*Aspergillus* sp.) |
| 7. | ALF-7 | 2.6 | 3 | Fluffy, white mycelium, slow growth, dark green sporulation at the centre (*Penicillium* sp.) |
| 8. | ALF-8 | 2.3 | 3 | Sparse white mycelium, heavy black sporulation at the centre, fast growth (*Aspergillus* sp.) |
| 9. | ALF-9 | 3.9 | 4 | White fluffy mycelium, black sporulation at the centre, fast growth (*Aspergillus* sp.) |
| 10. | ALF-10 | 4.0 | 5 | White, fluffy mycelium, dark green sporulation at the centre (*Penicillium* sp.). |
| 11. | ALF-11 | 2.4 | 3 | Dark green, flat mycelium, slow growth (*Penicillium* sp.) |
| 12. | ALF-12 | 4.0 | 5 | Sparse, white fluffy mycelium with dark green sporulation, slow growth (*Penicillium* sp.) |
| 13. | ALF-13 | 2.4 | 3 | Dark green sporulation, slow growth, brown metabolite in the medium (*Penicillium* sp.) |
| 14. | ALF-14 | 2.2 | 3 | White fluffy mycelium, black spores at the centre, very fast growth (*Aspergillus* sp.) |

| | | | | |
|---|---|---|---|---|
| 15. | ALF-15 | 3.7 | 4 | Sparse mycelium, sparse black spores in concentric pattern (*Aspergillus* sp.). |
| 16. | ALF-16 | 3.5 | 4 | White thick mycelium, olive green sporulation at the centre (*Aspergillus* sp.) |
| 17. | ALF-17 | 4.8 | 5 | White fluffy mycelium, heavy black sporulation at the centre ,fast growth (*A. niger*). |
| 18. | ALF-18 | 3.7 | 4 | White fluffy raised mycelium, thick olive green spores in concentric pattern (*A. flavus*) |
| 19. | ALF-19 | 3.2 | 4 | White fluffy mycelium, bluish green sporulation at the centre, slow growth (*Penicillium* sp.) |
| 20. | ALF-20 | 4.1 | 5 | Thin mycelium, sparse sporulation in concentric pattern from the centre , production of metabolite (*Aspergillus* sp.) |
| 21. | ALF-21 | 3.4 | 4 | White thick fluffy mycelium, olive green sporulation only at the centre (*A . flavus*.) |
| 22. | ALF-22 | 3.2 | 4 | White mycelium, black powdery sporulation (*Aspergillus* sp.) |
| 23. | ALF-23 | 3.6 | 4 | White mycelium, green powdery spores (*Penicillium* sp.) |
| 24. | ALF-24 | 2.9 | 3 | White mycelium, dark green sporulation (*Penicillium* sp.) |
| 25. | ALF-25 | 3.5 | 4 | White, thin fluffy mycelium with cream colour sporulation at the centre |
| 26. | ALF-26 | 4.2 | 5 | Yellowish brown mycelium with creamy yellow spores at the centre, fast growth (*Paceilomyces* sp.) |
| 27. | ALF-27 | 1.1 | 2 | White mycelium metabolite production, slow growth |
| 28. | ALF-28 | 4.5 | 5 | Black sporulation in concentric pattern, fast growth (*A. niger*) |
| 29. | ALF-29 | 3.7 | 4 | Brownish green coir like mycelia growth, no visible sporulation, fast growth |
| 30. | ALF-30 | 4.0 | 5 | Dark green powdery growth (*Penicillium* sp.) |
| **COIR PITH** | | | | |
| 31. | CLF-1 | 4.4 | 5 | Sparse,fluffy mycelium with black sporulation at the centre, fast growth , metabolite production (*Aspergillus* sp.) |

| | | | | |
|---|---|---|---|---|
| 32. | CLF-2 | 4.0 | 5 | white fluffy mycelium with black sporulation at the centre, fast growth (*A. niger*) |
| 33. | CLF-3 | 2.8 | 3 | White mycelium with dark green sporulation, slow growth (*Penicillium* sp.) |
| 34. | CLF-4 | 3.9 | 4 | White mycelium, with black spores, fast growth (*Aspergillus* sp.) |
| 35. | CLF-5 | 3.8 | 4 | White mycelium with black spores in the centre, fast growth (*Aspergillus* sp.) |
| 36. | CLF-6 | 3.9 | 4 | White mycelium, dark green sporulation in the centre, slow growth (*Penicillium* sp.) |
| 37. | CLF-7 | 4.0 | 5 | White, thin mycelium, black sporulation in the centre, fast growth (*A. niger*) |
| 38. | CLF-8 | 4.2 | 5 | White mycelium, black sporulation (*Aspergillus* sp.) |
| 39. | CLF-9 | 3.1 | 4 | White mycelium, green sporulation (*Penicillium* sp.) |
| 40. | CLF-10 | 4.1 | 5 | White mycelium, black sporulation (*A. niger*). |
| 41. | CLF-11 | 4.5 | 5 | White mycelium, brownish black sporulation in a concentric pattern, fast growth (*A. niger*) |
| 42. | CLF-12 | 3.1 | 4 | White mycelium, light green sporulation (different *Aspergillus* sp.) |
| 43. | CLF-13 | 3.5 | 4 | Dark green sporulation, yellow metabolite (*Penicillium* sp.) |
| 44. | CLF-14 | 5.0 | 5 | White fluffy mycelium, black sporulation, fast growth (*A. niger*) |
| 45. | CLF-15 | 3.9 | 4 | White mycelium, olive green sporulation in concentric pattern (*Aspergillus* sp.) |
| 46. | CLF-16 | 3.0 | 4 | White fluffy mycelium, green sporulation (*Penicillium* sp.) |
| 47. | CLF-17 | 3.6 | 4 | White mycelium olive green sporulation (*Aspergillus* sp.) |
| 48. | CLF-18 | 4.0 | 5 | White mycelium, black sporulation, fast growth (*A. niger*) |
| 49. | CLF-19 | 4.0 | 5 | White mycelium, olive green sporulation, dark green sporulation at the centre (*A . flavus*) |
| 50. | CLF-20 | 4.2 | 5 | White fluffy mycelium, black sporulation (*A. niger* ) |

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| 51. | CLF-21 | 3.0 | 4 | White mycelium, black sporulation in a concentric pattern, fast growth (*Aspergillus* sp.) |
| 52. | CLF-22 | 3.1 | 4 | White mycelium, brown sporulation, yellow metabolite (Different *Aspergillus* sp.) |
| 53. | CLF-23 | 4.1 | 5 | White mycelium, black sporulation in a concentric pattern, fast growth (*A. niger*) |
| 54. | CLF-24 | 4.5 | 5 | White raised mycelium, black sporulation (*A. niger*) |
| 55. | CLF-25 | 3.1 | 4 | White mycelium, olive green sporulation (*Aspergillus* sp.) |
| 56. | CLF-26 | 3.0 | 4 | White mycelium, black sporulation in a concentric pattern, fast growth (*Aspergillus* sp.) |
| 57. | CLF-27 | 4.2 | 5 | White mycelium, black sporulation in concentric pattern (*A. niger*) |
| 58. | CLF-28 | 1.9 | 2 | White mycelium, olive green sporulation (*Aspergillus* sp.) |
| 59. | CLF-29 | 3.8 | 4 | White mycelium, black sporulation (*Aspergillus* sp.) |
| 60. | CLF-30 | 1.9 | 2 | White mycelium, no visible sporulation |
| 61. | CLF-31 | 1.9 | 2 | White mycelium, olive green sporulation (*Aspergillus* sp.) |
| 62. | CLF-32 | 1.0 | 1 | White mycelium, no visible sporulation |
| 63. | CLF-33 | 5.1 | 5 | Brownish black sporulation. Yellow metabolite in the media (*Aspergillus* sp.) |
| 64. | CLF-34 | 4.4 | 5 | White mycelium, black sporulation, fast growth (*A. niger*) |
| 65. | CLF-35 | 2.2 | 3 | White mycelium, olive green sporulation (*Aspergillus* sp.) |
| 66. | CLF-36 | 3.9 | 4 | White mycelium, dark brown sporulation (*Aspergillus* sp.) |
| **ELEPHANT DUNG** | | | | |
| 67. | ELF-1 | 2.6 | 3 | White fluffy mycelium, green sporulation (*Penicillium* sp.) |
| 68. | ELF-2 | 2.5 | 3 | White mycelium, olive green sporulation (*Aspergillus* sp.). |
| 69. | ELF-3 | 4.0 | 5 | White mycelium, black sporulation (*A. niger*) |
| 70. | ELF-4 | 1.7 | 2 | White fluffy mycelium, dark green sporulation at the centre, slow growth (*Penicillium* sp.) |

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| 71. | ELF-5 | 2.6 | 3 | White mycelium, black sporulation, fast growth (*Aspergillus* sp.) |
| 72. | ELF-6 | 2.5 | 3 | White mycelium, black sporulation (*Aspergillus* sp.) |
| 73. | ELF-7 | 2.3 | 3 | White mycelium, black sporulation, fast growth (*Aspergillus* sp.) |
| 74. | ELF-8 | 4.1 | 5 | Brownish black sporulation in concentric pattern, yellow metabolite in media, fast growth (*A. niger*). |
| 75. | ELF-9 | 1.7 | 2 | White mycelium, no visible sporulation. |
| 76. | ELF-10 | 2.5 | 3 | Dark green sporulation in concentric pattern |
| 77. | ELF-11 | 2.4 | 3 | Black sporulation, light yellow metabolite in the media (*Aspergillus* sp.) |
| 78. | ELF-12 | 1.2 | 2 | White mycelium, black sporulation (*Aspergillus* sp.) |
| 79. | ELF-13 | 3.5 | 4 | White mycelium, green sporulation (*Penicillium* sp.) |
| **FOREST AREA** | | | | |
| 80. | FLF-1 | 3.9 | 4 | Fluffy white mycelium with black sporulation at the centre, fast growth (*Aspergillus* sp.) |
| 81. | FLF-2 | 3.1 | 4 | White mycelium, dark green sporulation at the centre, dark brown metabolite (*Penicillium* sp.) |
| 82. | FLF-3 | 3.4 | 4 | White mycelium, black sporulation in concentric pattern (*Aspergillus* sp.) |
| 83. | FLF-4 | 3.3 | 4 | White fluffy mycelium, olive green sporulation in concentric pattern (*Aspergillus* sp.) |
| 84. | FLF-5 | 4.0 | 5 | Thin, white mycelium, black spores in concentric pattern, fast growth ,yellow metabolite (*Aspergillus* sp.). |
| 85. | FLF-6 | 2.8 | 3 | Dark green sporulation in white mycelium, slow growth |
| 86. | FLF-7 | 2.4 | 3 | White mycelium, black sporulation, yellow metabolite production |
| 87. | FLF-8 | 4.7 | 5 | White fluffy mycelium, black sporulation, brown metabolite in the media (*A. niger*) |

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| **CASHEW PLANTATION** | | | | |
| 88. | CaLF-1 | 3.1 | 4 | White mycelium, heavy bluish green sporulation at the centre (*Penicillium* sp.) |
| 89. | CaLF-2 | 3.2 | 4 | White fluffy mycelium, sparse black sporulation, fast growth (*Aspergillus* sp.) |
| 90. | CaLF-3 | 3.8 | 4 | White mycelium, heavy olive green sporulation (*Aspergillus* sp.) |
| 91. | CaLF-4 | 4.2 | 5 | White fluffy mycelium, fast growth. Heavy black sporulation (*A. niger*) |
| 92. | CaLF-5 | 4.1 | 5 | Thin, white mycelium, dark green sporulation (*Penicillium* sp.) |
| 93. | CaLF-6 | 2.7 | 3 | Thin mycelium, dark bluish green spores, slow growth (*Penicillium* sp.) |
| 94. | CaLF-7 | 4.1 | 5 | White mycelium, heavy black sporulation, white scleroita at the centre, fast growth (different *Aspergillus* sp.) |
| 95. | CaLF-8 | 3.4 | 4 | White thin mycelium, olive green sporulation (*Aspergillus* sp.) |
| 96. | CaLF-9 | 3.1 | 4 | Thin white mycelium. Heavy dark green sporulation, slow growth (*Aspergillus* sp.) |
| 97. | CaLF-10 | 3.1 | 4 | Thin white mycelium, creamish yellow sporulation at the centre, slow growth |
| **TEAK PLANTATION** | | | | |
| 98. | TLF-1 | 4.1 | 5 | White mycelium, heavy black sporulation, fast growth (*A. niger*) |
| 99. | TLF-2 | 3.2 | 4 | White mycelium, heavy olive green sporulation (*Aspergillus* sp.). |
| 100. | TLF-3 | 3.1 | 4 | White fluffy mycelium, black sporulation (*Aspergillus* sp.) |
| 101. | TLF-4 | 3.3 | 4 | White fluffy mycelium, olive green sporulation in concentric pattern (*Aspergillus* sp.) |
| 102. | TLF-5 | 3.4 | 4 | White mycelium, yellowish white spore masses and sparse black spores at the centre |
| 103. | TLF-6 | 3.3 | 4 | White mycelium, black sporulation, fast growth (*Aspergillus* sp.) |

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| 104. | TLF-7 | 2.8 | 3 | White mycelium, brownish black sporulation (*Aspergillus* sp.) |
| 105. | TLF-8 | 3.2 | 4 | Bright yellow mycelium, white sporulation |
| | | **MANGO ORCHARD** | | |
| 106. | MLF-1 | 4.0 | 5 | White fluffy mycelium, sparse black sporulation, brown metabolite, fast growth (*Aspergillus* sp.) |
| 107. | MLF-2 | 2.4 | 3 | White mycelium, dark green sporulation slow growth (*Penicillium* sp.) |
| 108. | MLF-3 | 4.1 | 3 | White fluffy mycelium, black concentric sporulation, fast growth and brown metabolite (*Aspergillus* sp.) |
| 109. | MLF-4 | 2.0 | 2 | White mycelium, dark green spores at the centre, slow growth (*Penicillium* sp.) |
| 110. | MLF-5 | 4.0 | 5 | White mycelium, heavy black sporulation, fast growth (*Aspergillus* sp.) |
| 111 | MLF-6 | 3.5 | 4 | Thin white spreading mycelium ,yellowish green spore mass |
| 112. | MLF-7 | 4.2 | 5 | White mycelia, heavy black sporulation. Fast growth (*A. niger*) |
| | | **COMPOST PIT** | | |
| 113. | CpLF-1 | 4.0 | 5 | White fluffy mycelium, thick black spores at the centre, very fast growth (*A. niger*) |
| 114. | CpLF-2 | 3.5 | 4 | White fluffy mycelium with dark green sporulation at the centre |
| 115. | CpLF-3 | 4.0 | 5 | White, fluffy raised mycelium with concentric olive green sporulation at the centre, very fast growth (*A. niger* ) |
| 116. | CpLF-4 | 3.6 | 4 | White fluffy raised mycelium with dark olive green sporulation at the centre (*Aspergillus* sp.) |
| 117. | CpLF-5 | 2.8 | 3 | Fluffy mycelium, dark green spores at the centre, slow growth (*Penicillium* sp.) |
| | | **VERMI COMPOST** | | |
| 118. | VeLF-1 | 4.5 | 5 | White mycelium, black sporulation, fast growth (*A. niger*) |

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| 119. | VeLF-2 | 3.3 | 4 | White mycelium with dark olive green sporulation at the centre (*Aspergillus* sp.) |
| **SPENT MUSHROOM SUBSTRATE** | | | | |
| 120. | SMLF-1 | 3.7 | 4 | White fluffy raised mycelium with, olive green sporulation at the centre, fast growth (*A. flavus*.) |
| 121. | SMLF-2 | 4.0 | 5 | White fluffy raised mycelium with, olive green sporulation at the centre, fast growth (*A. flavus*) |
| 122. | SMLF-3 | 2.3 | 3 | White mycelium, black sporulation |
| 123. | SMLF-4 | 3.6 | 4 | White mycelium, black sporulation in concentric pattern (*Aspergillus* sp.) |
| **SAW MILL** | | | | |
| 124. | SLF-1 | 4.1 | 5 | White mycelium, black sporulation, fast growth (*A. niger* ) |
| 125. | SLF-2 | 4.1 | 5 | White mycelium, black sporulation, fast growth (*A. niger*) |
| 126. | SLF-3 | 3.0 | 4 | White mycelium, black sporulation in concentric pattern (*Aspergillus* sp.) |
| **COW DUNG** | | | | |
| 127. | CdLF-1 | 2.1 | 3 | White mycelium with dark green sporulation, slow growth |
| **BACTERIA** | | | | |
| 128 | ALB-1 | 1.8 | 4 | Milky white, slimy, fluidal colonies |
| 129 | ALB-2 | 2.3 | 3 | White, dry colonies with wavy margins |
| 130 | SLB-1 | 1.2 | 2 | Cream colour, slimy |
| 131 | SLB-2 | 1.3 | 2 | Milky white, slimy |
| 132 | SLB-3 | 1.3 | 2 | Yellow colour, dry colony |
| **ACTINOMYCETES** | | | | |
| 133 | ALA-1 | 2.8 | 3 | White, dry growth |
| | | | | |

*Mean of two replications

**1-5 Scale score chart**

| Score | Diameter of diffusion zone (cm) |
|---|---|
| 1 | < 1 |
| 2 | 1- 2 |
| 3 | 2- 3 |
| 4 | 3 -4 |
| 5 | > 4 |

## Table 10. *In vitro* evaluation of tannin degraders

| Sl No. | Isolate No. | * Diameter of diffusion zone (cm) | Score | Colony characters |
|---|---|---|---|---|
| AYURVEDIC PHARMACEUTICAL UNITS | | | | |
| FUNGI | | | | |
| 1. | ATF-1 | 3.7 | 4 | Thin mycelium, sparse sporulation, fast gowth (*Aspergillus* sp.) |
| 2. | ATF-2 | 1.7 | 2 | White mycelium, black sporulation in concentric pattern (*Aspergillus* sp.) |
| 3. | ATF-3 | 4.0 | 5 | Thin white fluffy mycelium with spore masses in concentric pattern (*A. niger.*) |
| 4. | ATF-4 | 2.4 | 3 | Thin white mycelium sparse black sporulation (*Aspergillus* sp.) |
| 5. | ATF-5 | 2.3 | 3 | Green powdery growth (*Penicillium* sp.). |
| 6. | ATF-6 | 3.9 | 4 | Thin white mycelium black sporulation at the centre (*Aspergillus* sp.) |
| 7. | ATF-7 | 4.0 | 5 | White mycelium, black sporulation, fast growth (*A. niger*) |
| 8. | ATF-8 | 3.9 | 4 | Thin white mycelium black sporulation at the centre (*Aspergillus* sp.) |
| 9. | ATF-9 | 3.3 | 4 | White mycelium, dark green sporulation at the centre (*Penicillium* sp.) |
| 10. | ATF-10 | 2.4 | 3 | White fluffy mycelium, black sporulation (*Aspergillus* sp.) |
| 11. | ATF-11 | 2.1 | 3 | White mycelium, olive green sporulation ((*Aspergillus* sp.) |
| 12. | ATF-12 | 3.5 | 4 | White fluffy raised mycelium, no sporulation |
| 13. | ATF- 13 | 4.0 | 5 | Thin white mycelium, sparse sporulation, fast growth |
| 14. | ATF-14 | 3.7 | 4 | White fluffy mycelium with sparse black sporulation at the centre (*Aspergillus* sp.) |
| 15. | ATF- 15 | 4.4 | 5 | Thin white mycelium, black sporulation at centre,fast growth (*A. niger*) |
| 16. | ATF-16 | 4.0 | 5 | Thin white mycelium, black sporulation at the centre, fast growth (*A. niger*) |

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|---|---|---|---|---|
| 17. | ATF-17 | 3.4 | 4 | Thin white mycelium, black sporulation at the centre, fast growth (*Aspergillus* sp.) |
| 18. | ATF-18 | 2.1 | 3 | White mycelium, green sporulation (*Penicillium* sp.) |
| 19. | ATF-19 | 4.5 | 4 | Thin white mycelium, black sporulation at the centre , yellow metabolite (*Aspergillus* sp.) |
| 20. | ATF-20 | 3.5 | 4 | White mycelium, green sporulation (*Penicillium* sp.) |
| 21. | ATF-21 | 3.2 | 4 | Thin white mycelium, dark green sporulation at the centre, yellowish brown metabolite (*Penicillium* sp.). |
| 22. | ATF-22 | 3.4 | 4 | White cottony mycelium dark green sporulation, slow growth (*Aspergillus* sp.) |
| 23. | ATF-23 | 4.0 | 5 | Thin white mycelium, black sporulation in concentric pattern (*A. niger*) |
| 24. | ATF-24 | 4.0 | 5 | Thin white mycelium with brownish green sporulation at the centre (*A. niger* ) |
| 25. | ATF-25 | 3.8 | 4 | Dark bluish green powdery spores, black discoloration on the media (*Penicillium* sp.) |
| **COIR PITH** | | | | |
| 26. | CTF-1 | 3.9 | 4 | White mycelium, black spores (*Aspergillus* sp.) |
| 27. | CTF-2 | 4.0 | 5 | White fluffy mycelium, olive green sporulation (*A. flavus*) |
| 28. | CTF-3 | 4.0 | 5 | White fluffy mycelium with black sporulation at the centre, fast growth (*A. niger*) |
| 29. | CTF-4 | 4.0 | 5 | White fluffy mucelium, green sporulation (different *Aspergillus* sp.) |
| 30. | CTF-5 | 3.6 | 4 | White mycelium,green sporulation (*Penicillium* sp.) |
| 31. | CTF-6 | 3.5 | 4 | White fluffy mycelium, black sporulation (*Aspergillus* sp.) |
| 32. | CTF-7 | 3.5 | 4 | White fluffy mycelium, black sporulation (*Aspergillus* sp.) |
| 33. | CTF-8 | 4.1 | 5 | White mycelium, black sporulation, fast growth ( *A. niger* ) |
| 34. | CTF-9 | 4.1 | 5 | White fluffy mycelium, olive green sporulation (*A. flavus*) |
| 35. | CTF-10 | 3.2 | 4 | White mycelium, green sporulation, slow growth ( *Penicillium* sp.) |

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| 36. | CTF-11 | 1.9 | 2 | White mycelium, olive green powdery growth (*Aspergillus* sp.) |
| 37. | CTF-12 | 4.3 | 5 | White fluffy mucelium, green sporulation in centre of the plate (*A. flavus*) |
| 38. | CTF-13 | 2.9 | 3 | White mycelium,black sporulation in concentric pattern (*Aspergillus* sp.) |
| 39. | CTF-14 | 4.1 | 5 | White mycelium, black sporulation, fast growth (*A. niger*) |
| 40. | CTF-15 | 3.5 | 4 | White mycelium, black sporulation, fast growth (*Aspergillus* sp.) |
| 41. | CTF-16 | 2.3 | 3 | White mycelium, green sporulation (*Aspergillus* sp.) |
| 42. | CTF-17 | 5.0 | 5 | White mycelium, black sporulation, fast growth (*A. niger*) |
| 43. | CTF-18 | 4.6 | 5 | White mycelium, black sporulation, fast growth (*A. niger*) |
| 44. | CTF-19 | 3.2 | 4 | White mycelium,olive green sporulation, dark green sporulation at the centre (*A.flavus*) |
| 45. | CTF-20 | 4.3 | 5 | White mycelium,black sporulation, fast growth (*A. niger*) |
| 46. | CTF-21 | 3.1 | 4 | White fluffy mycelium, olive green sporulation (*Aspergillus* sp.) |
| 47. | CTF-22 | 3.3 | 4 | White mycelium, olive green sporulation at the centre (*Aspergillus* sp.) |
| 48. | CTF-23 | 3.8 | 4 | White mycelium, brown powdery growth, brown discolouration on the reverse side |
| 49. | CTF-24 | 4.1 | 5 | White raised mycelium,black sporulation (*A. niger*) |
| 50. | CTF-25 | 5.5 | 5 | White raised mycelium, black sporulation (*A. niger*.) |
| 51. | CTF-26 | 3.1 | 4 | White mycelium, black sporulation, fast growth (*Aspergillus* sp.) |
| 52. | CTF-27 | 2.5 | 3 | White fluffy mycelium, black sporulation in concentric pattern (*Aspergillus* sp.) |
| 53. | CTF-28 | 2.6 | 3 | White mycelium, olive green sporulation (*Aspergillus* sp.) |
| 54. | CTF-29 | 1.9 | 2 | White mycelium, green sporulation (*Penicillium* sp.) |

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| 55. | CTF-30 | 2.3 | 3 | White raised mycelium, black sporulation (*Aspergillus* sp.) |
| 56. | CTF-31 | 2.6 | 3 | White mycelium, olive green sporulation at the centre (*Aspergillus* sp.) |
| 57. | CTF-32 | 3.6 | 4 | White raised mycelium, black sporulation (*Aspergillus* sp.). |
| 58. | CTF-33 | 5.2 | 5 | White mycelium, brownish black sporulation (*A. niger*) |
| **ELEPHANT DUNG** | | | | |
| 59. | ETF-1 | 4.6 | 5 | Thin white mycelium with brownish green sporulation at the centre (*A. niger* ) |
| 60. | ETF-2 | 3.0 | 4 | White mycelium, no sporulation. |
| 61. | ETF-3 | 2.3 | 3 | White mycelium, black sporulation in concentric pattern (*Aspergillus* sp.) |
| 62. | ETF-4 | 2.2 | 3 | White mycelium, black sporulation in a concentric pattern, fast growth (*Aspergillus* sp.) |
| 63. | ETF-5 | 3.3 | 4 | White mycelium, black sporulation (*Aspergillus* sp.) |
| 64. | ETF-6 | 2.1 | 3 | White mycelium, no sporulation |
| 65. | ETF-7 | 2.9 | 3 | White mycelium, no sporulation |
| 66. | ETF-8 | 2.1 | 3 | Grey, thin powdery growth, reverse side turns black |
| 67. | ETF-9 | 2.3 | 3 | White mycelium,black sporulation in concentric pattern (*Aspergillus* sp.) |
| 68. | ETF-10 | 3.1 | 4 | White mycelium, brownish black sporulation in a concentric pattern, fast growth (*Aspergillus* sp.) |
| 69. | ETF-11 | 3.2 | 4 | White mycelium, black sporulation in concentric pattern (*Aspergillus* sp.) |
| 70. | ETF-12 | 3.2 | 4 | White mycelium, black sporulation (*Aspergillus* sp.) |
| **FOREST AREA** | | | | |
| 71. | FTF-1 | 3.5 | 4 | Very fast growth, black sparse sporulation on white mycelium (*Aspergillus* sp.) |
| 72. | FTF-2 | 2.4 | 3 | Dark green sporulation on white mycelium, slow growth (*Penicillium* sp.) |

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|---|---|---|---|---|
| 73. | FTF-3 | 5.0 | 5 | White mycelium, black thick sporulation at the centre, yellow metabolite in the media, fast growth (*A. niger*) |
| 74. | FTF-4 | 4.0 | 5 | Thin white fluffy mycelium, sparse black spores at the centre, fast growth (*A. niger*) |
| 75. | FTF-5 | 3.8 | 4 | Dark brown concentric sporulation, fast growth (*Aspergillus* sp.) |
| 76. | FTF-6 | 2.5 | 3 | White mycelium with a floral design concentric growth, dark green spores at the centre, slow growth (*Penicillium* sp.) |
| 77. | FTF-7 | 2.6 | 3 | White mycelium with black sporulation at the centre (*Aspergillus* sp.) |
| 78. | FTF-8 | 3.4 | 4 | White mycelium, black sporulation, yellow metabolite in the media (*Aspergillus* sp.) |
| 79. | FTF-9 | 2.3 | 3 | Thin white mycelium, black sporulation in concentric pattern (*Aspergillus* sp.) |
| 80. | FTF-10 | 3.2 | 4 | White fluffy mycelium, black sporulation, yellow metabolite (*Aspergillus* sp.) |
| **CASHEW PLANTATION** | | | | |
| 81 | CaTF-1 | 4.0 | 5 | Thin white fluffy mycelim with black sporulation in concentric zones (*A. niger*) |
| 82. | CaTF-2 | 3.2 | 4 | White fluffy mycelium, very slow growth, no sporulation |
| 83. | CaTF-3 | 3.8 | 4 | White fluffy mycelium with sparse black sporulation at the centre (*Aspergillus* sp.) |
| 84. | CaTF-4 | 3.0 | 4 | White mycelium, dark green spores at the centre, slow growth (*Penicillium* sp.) |
| 85. | CaTF-5 | 4.0 | 5 | Thin white mycelium, black sporulation in concentric pattern (*A. niger*) |
| 86. | CaTF-6 | 3.0 | 4 | Dark green powdery sporulation blackish discoloration of the media (*Penicillium* sp.) |
| 87. | CaTF-7 | 3.4 | 4 | Light green powdery sporulation greenish black discoloration of the media (*Penicillium* sp.)- indication of polyphenol oxidase activity |
| 88. | CaTF-8 | 3.7 | 4 | Thin white mycelium, black sporulation (*Aspergillus* sp.) |

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|---|---|---|---|---|
| 89. | CaTF-9 | 3.1 | 4 | Thin white mycelium, light olive green sporulation, slow growth (*Aspergillus* sp.) |
| **TEAK PLANTATION** | | | | |
| 90. | TTF-1 | 3.5 | 4 | Thin white fluffy mycelium, sparse black sporulation at the centre (*Aspergillus* sp.) |
| 91. | TTF-2 | 3.0 | 4 | White mycelium, yellowish green sporulation at the centre (*Aspergillus* sp.) |
| 92. | TTF-3 | 4.6 | 5 | White fluffy raised mycelium, brown spores later turning black, yellow metabolite in the media, fast growth (*A. niger*) |
| 93. | TTF-4 | 3.7 | 4 | White fluffy mycelium, no visible sporulation |
| 94. | TTF-5 | 3.2 | 4 | White fluffy mycelium with black sporulation at the centre, fast growth (*Aspergillus* sp.) |
| 95. | TTF-6 | 3.5 | 4 | White fluffy mycelium, olive green sporulation (*Aspergillus* sp.) |
| 96. | TTF-7 | 3.7 | 4 | White mycelium, olive green powdery growth (*A. flavus.*) |
| 97. | TTF-8 | 3.3 | 4 | White mycelium, black sporulation, golden coloured powdery growth at the centre (*Aspergillus* sp.) |
| **MANGO ORCHARD** | | | | |
| 98. | MTF-1 | 4.0 | 5 | Brownish black sporulation in concentric pattern., fast growth (*Aspergillus* sp.) |
| 99. | MTF-2 | 2.8 | 3 | Greyish white coir like mycelium, dark discolouration on the reverse side, black sporulation only at the centre (*Aspergillus* sp.) |
| 100. | MTF-3 | 3.5 | 4 | White thin mycelium, with dark green sporulation in concentric pattern (*Penicillium* sp.) |
| 101. | MTF-4 | 3.4 | 4 | Thin white mycelium with dark green sporulation at the centre (*Penicillium* sp.) |
| 102. | MTF-5 | 4.8 | 5 | White fluffy mycelium, black sporulation, fast growth (*A. niger*) |
| 103. | MTF-6 | 4.7 | 5 | Brownish black sporulation in concentric pattern, fluffy white mycelium, fast growth (*A. niger.*) |

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|---|---|---|---|---|
| 104. | MTF-7 | 3.5 | 4 | White mycelial growth in concentric pattern, slow growth |
| 105. | MTF-8 | 3.7 | 4 | White fluffy mycelium, with sparse black sporulation in concentric pattern, fast growth |
| 106 | MTF-9 | 3.8 | 4 | White fluffy mycelium, heavy black sporulation, fast growth (*Aspergillus* sp.) |
| 107. | MTF-10 | 2.8 | 3 | White mycelial growth in concentric pattern, slow growth (*Penicillium* sp.) |
| **COMPOST PIT** | | | | |
| 108. | CpTF-1 | 3.7 | 4 | Thin white mycelium, black sporulation, fast growth (*Aspergillus* sp.) |
| 109. | CpTF-2 | 4.0 | 5 | Thin white mycelium sparse sporulation at the centre, fast growth (*A. niger*.) |
| 110. | CpTF-3 | 3.6 | 4 | Thin white fluffy mycelium, olive green sporulation at the centre (*Aspergillus* sp.) |
| **SPENT MUSHROOM SUBSTRATE** | | | | |
| 111. | SMTF-1 | 4.0 | 5 | White mycelium, black sporulation at the centre (*A. niger*.) |
| 112. | SMTF-2 | 3.1 | 4 | White mycelium with dark green sporulation (*Penicillium* sp.) |
| 113. | SMTF-3 | 4.0 | 5 | White mycelium black sporulation. fast growth (*A. niger*) |
| 114. | SMTF-4 | 3.1 | 4 | White mycelium, black sporulation (*Aspergillus* sp.) |
| 115. | SMTF-5 | 2.8 | 3 | White mycelium, no sporulation |
| **SAW MILL** | | | | |
| 116. | STF-1 | 3.9 | 4 | White mycelium, black sporulation(*Aspergillus* sp.) |
| 117. | STF-2 | 2.5 | 3 | White mycelium, green sporulation (*Penicillium* sp.) |
| **COW DUNG** | | | | |
| 118. | CdTF-1 | 2.4 | 3 | White mycelium, no sporulation, slow growth |
| 119. | CdTF-2 | 4.0 | 5 | White fluffy mycelium with black sporulation at the centre (*A. niger*.) |
| **YEAST** | | | | |
| 120. | ATY-1 | 2.5 | 3 | Cream slimy growth |

*Mean of two replications

**1-5 Scale Score chart**

| Score | Diameter of diffusion zone (cm) |
|---|---|
| 1 | < 1 |
| 2 | 1- 2 |
| 3 | 2- 3 |
| 4 | 3 - 4 |
| 5 | > 4 |

# PLATE 3

## *IN VITRO* EVALUATION OF MICROBIAL DEGRADERS

### A. Cellulose Degraders

ECF -2 (Elephant dung)

CdCB-1 (Fresh cow dung)

CaCA-3 (Cashew litters)

### B. Lignin degraders

ALF -26 (Ayurvedic waste)

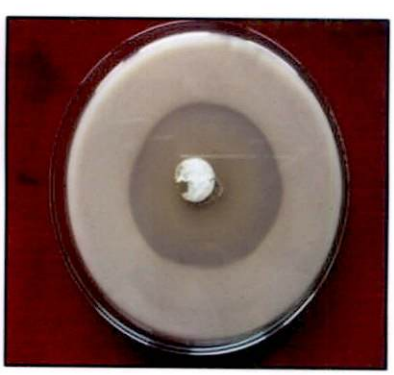
CLF- 33 (Coir pith)

TLF -8 (Teak litters)

### C. Tannin Degraders

ATF-19 (Ayurvedic waste)

FTF-10 (Forest area)



CaTF- 7 (Cashew litter)

4.0 - 5.5 cm. Degraders from cow dung, vermicompost, and saw dust spent mushroom substrate dumping site also showed diffusion zones of 4.0 cm.

In addition to the diameter of diffusion zones, type of organisms, metabolite production, peculiarity of colony characters, especially for the *Aspergillus* spp. were also considered for the selection of degraders. Based on these facts, cellulose degraders such as ACF-6, ECF-2, CCF-4, TCF-5, ACB-2, CCB-8, CdCB-1, ACA-5, ECA-8, CaCA-3, CaCA-5 and TmCA-2, lignin degraders ALF-10; ALF-19, ALF-20, ALF-30, CLF-1, CLF-6, CLF-12, CLF-13, CLF-16, CLF-22, CLF-36, CaLF-5, CaLF-7, MLF-1, TLF-8, ALB-2 and ALA-1, tannin degraders, ATF-19, CTF- 4, CaTF-7, FTF-10, ATY-1 were selected for further studies.

Summing up the above results, it is observed that, samples from ayurvedic wastes showed most efficient degraders of cellulose, lignin and tannin components. In addition, the samples from coconut by product dumping sites also yielded effective lignin and tannin degraders. Of the three types, fungi were the most efficient organisms for the degradation of all three components. The fungal isolate, ACF-4, obtained from *kashayam* waste from SD pharmacy Alappuzha, and TCF-5 from Peechi teak plantation were the most efficient cellulose degraders. In case of lignin-tannin degraders, fungi isolated from coir pith dumping sites of Andiyoor, recorded maximum diffusion zones. *Aspergillus* and *Penicillium* spp. were found to be the predominant degraders of cellulose, lignin and tannin components and *Aspergillus* spp. was the most efficient degrader.

## 4.4. CROSS DEGRADING ABILITY OF MICROBIAL DEGRADERS ON VARIOUS SELECTIVE MEDIA

Results of the *in vitro* screening on cross degradability of selected microbial degraders presented in Tables 12, 13 and 14 showed that, all tested degraders had the ability to degrade all the three chemical components *viz.* cellulose, lignin and tannin.

It is observed from the Table 12 that, all cellulose degraders showed maximum diffusion zones on their respective medium which ranged from 2.5 - 6.0 cm, whereas, the zones produced on lignin and tannin media were only 1.5 - 3.4 cm and 2.0 - 4.0 cm respectively.

In case of lignin degraders, fifteen isolates showed maximum zones on their respective selective medium, ranged from 2.3 - 4.4 cm whereas, the isolates, ALF-19 and TLF-8 recorded maximum zone of 4.8 cm on tannin medium and CLF-12 on cellulose medium (3.5 cm) as compared to their selective lignin medium (Table 13).

The diffusion zones of tannin degraders on their respective media ranged from 2.5 – 4.5 cm. But CaTF-7 and FTF -10 were good degraders of other two components recording maximum zones of 5.0 and 5.7 cm and 4.0 and 4.4 cm on lignin and cellulose media respectively (Table 14).

## 4.5. *IN VITRO* EVALUATION OF SELECTED DEGRADERS RESPECTIVE HOST SUBSTRATES

Thirty three degraders selected, based on the size of diffusion zones, cross degradability, type and species of organisms were tested on their respective host substrates in conical flasks and plastic pots under lab condition and the results are presented in Table 15, which showed that, in both cases, composting periods were found almost same which ranged from 40 - 125 days depending on degraders and different substrates used (Plate 4 A).

With respect to ayurvedic waste, the composting period ranged from 40 - 45 days. Among the 10 ayurvedic degraders, six isolates (ACF-6, ALF-26, ACB-2, ACA -5, ALA-1 and ATY -1) showed early maturity at 40 days and other four recorded 45 days for degradation of the substrate.

Coir pith degraders *viz.* CCF-4, CLF-6, CLF-12, CLF-13 and CCB-8 recorded 43 days and others showed 45- 47 days for composting. Both degraders

**Table 12 . Cross degrading ability of cellulose degraders on lignin and tannin media**

| Sl. No. | Cellulose degrader | * Diamteter of diffusion zone (cm) | | |
|---|---|---|---|---|
| | | Cellulose | Lignin | Tannin |
| 1 | ACF-6 | 4.8 | 3.0 | 3.1 |
| 2 | ECF-2 | 5.5 | 3.3 | 3.0 |
| 3 | CCF-4 | 2.7 | 1.5 | 2.6 |
| 4 | TCF-5 | 6.0 | 3.2 | 3.2 |
| 5 | ACB-2 | 5.5 | 3.4 | 3.0 |
| 6 | CCB-8 | 5.5 | 3.3 | 4.0 |
| 7 | CdCB-1 | 5.7 | 3.3 | 3.2 |
| 8 | ACA-5 | 3.7 | 3.2 | 2.9 |
| 9 | ECA-8 | 2.9 | 2.1 | 2.0 |
| 10 | CaCA-3 | 4.2 | 2.1 | 2.0 |
| 11 | CaCA-5 | 3.2 | 2.5 | 2.1 |
| 12 | TmCA-2 | 3.3 | 1.8 | 2.1 |

* Mean of three replications

**Table 13 . Cross degrading ability of lignin degraders on cellulose and tannin media**

| Sl. No. | Lignin degraders | *Diameter of diffusion zone (cm) | | |
|---|---|---|---|---|
| | | Lignin | Tannin | Cellulose |
| 1 | ALF-10 | 4.0 | 3.1 | 2.1 |
| 2 | ALF-19 | 3.2 | 4.8 | 2.8 |
| 3 | ALF-20 | 4.1 | 4.1 | 3.0 |
| 4 | ALF-26 | 4.2 | 3.1 | 3.0 |
| 5 | ALF-30 | 4.0 | 3.3 | 2.1 |
| 6 | CLF-1 | 4.4 | 3.3 | 3.3 |
| 7 | CLF-6 | 3.9 | 3.2 | 3.5 |
| 8 | CLF-12 | 3.1 | 3.6 | 3.5 |
| 9 | CLF-13 | 3.5 | 3.0 | 3.4 |
| 10 | CLF-16 | 3.0 | 3.5 | 2.9 |
| 11 | CLF-22 | 3.1 | 2.8 | 3.0 |
| 12 | CLF-36 | 3.9 | 3.1 | 3.0 |
| 13 | CaLF-5 | 4.1 | 3.9 | 2.8 |
| 14 | CaLF-7 | 4.1 | 2.3 | 2.9 |
| 15 | MLF-1 | 4.0 | 4.0 | 3.4 |
| 16 | TLF-8 | 3.2 | 4.8 | 3.3 |
| 17 | ALB-2 | 2.3 | 1.6 | 1.0 |
| 18 | ALA-1 | 2.8 | 1.2 | 1.4 |

* Mean of three replications

**Table 14 .Cross degrading ability of tannin degraders on cellulose and lignin media**

| Sl. No. | Tannin degraders | *Diameter of diffusion zone (cm) | | |
|---|---|---|---|---|
| | | Tannin | Lignin | Cellulose |
| 1 | ATF-19 | 4.5 | 3.9 | 3.7 |
| 2 | CTF-4 | 4.0 | 4.0 | 3.8 |
| 3 | CaTF-7 | 3.4 | 5.0 | 5.7 |
| 4 | FTF-10 | 3.2 | 4.0 | 4.4 |
| 5 | ATY-1 | 2.5 | 1.6 | 1.7 |

*Mean of three replications

**Table 15. *In vitro* evaluation of selected degraders on respective host substrate**

| Sl. No. | Isolates | *Days for maturity | Substrate degraded |
|---|---|---|---|
| 1 | ACF-6 | 40 | Ayurvedic waste |
| 2 | ALF-20 | 45 | Ayurvedic waste |
| 3 | ALF-26 | 40 | Ayurvedic waste |
| 4 | ALF-30 | 45 | Ayurvedic waste |
| 5 | ATF-19 | 45 | Ayurvedic waste |
| 6 | ACB-2 | 40 | Ayurvedic waste |
| 7 | ALB-2 | 45 | Ayurvedic waste |
| 8 | ATY-1 | 40 | Ayurvedic waste |
| 9 | ACA-5 | 40 | Ayurvedic waste |
| 10 | ALA-1 | 40 | Ayurvedic waste |
| 11 | CCF-4 | 43 | Coir pith |
| 12 | CLF-1 | 46 | Coir pith |
| 13 | CLF-6 | 43 | Coir pith |
| 14 | CLF-12 | 43 | Coir pith |
| 15 | CLF-13 | 43 | Coir pith |
| 16 | CLF-16 | 45 | Coir pith |
| 17 | CLF-22 | 45 | Coir pith |
| 18 | CLF-36 | 47 | Coir pith |
| 19 | CTF-4 | 45 | Coir pith |
| 20 | CCB-8 | 43 | Coir pith |
| 21 | ECF-2 | 43 | Elephant dung |
| 22 | ECA-8 | 43 | Elephant dung |
| 23 | CaLF-5 | 75 | Cashew |
| 24 | CaLF-7 | 75 | Cashew |
| 25 | CaTF-7 | 75 | Cashew |
| 26 | CaCA-3 | 75 | Cashew |
| 27 | CaCA-5 | 75 | Cashew |
| 28 | MLF-1 | 80 | Mango |
| 29 | FTF-10 | 85 | Teak |
| 30 | TLF-8 | 60 | Teak |
| 31 | TCF-5 | 60 | Teak |
| 32 | CdCB-1 | 100 | Paddy straw |
| 33 | TmCA-2 | 125 | Paddy straw |

* Mean of three replications

of elephant dung, ECF-2 and ECA-8 showed degradation of the respective substrate by 43 days.

Among the leaf litter degraders, two degraders (TCF-5 and TLF-8) from teak showed lowest period of 60 days whereas, those from cashew and mango took 75 and 80 days for degradation.

The isolates, TmCA-2 from termite mound and CdCB-1 from fresh cow dung showed longer periods of 120 and 125 days for the composting of paddy straw.

## 4.6. *IN VIVO* SCREENING OF SELECTED DEGRADERS ON RESPECTIVE HOST SUBSTRATES

Degradability of twenty two degraders selected from *in vitro* studies were tested on their respective hosts under *in vivo* condition and the result is presented in Table 16. It is noted that, the maturity periods recorded for ayurvedic waste, coir pith and teak leaves were almost the same as that of lab condition (Plate 4 B).

Among the various degraders tested, isolates ALF-26, CLF-13, ECF-2 and ECA-8, TLF-8, and CaLF-5 showed minimum maturity periods of 45, 45, 50, 60 and 65 days for ayurvedic waste, coir pith, elephant dung, teak and cashew leaves respectively.

## 4.7. *IN VIVO* SCREENING OF SELECTED DEGRADERS ON OTHER HOST SUBSTRATES

From Table 17, it is noticed that, all selected 22 degraders isolated from their respective host substrates were capable of degrading other lignin-tannin rich plant residues which showed period of composting ranged from 45 - 50 days for ayurvedic waste and elephant dung, 45 - 55 days for coir pith. All leaf litters showed longer maturity periods, 60 - 65 days for teak, 65 - 70 days for mango and cashew leaves and aerobic composting of fodder grass showed 50 - 55 days for maturity.

Among the various degraders, minimum composting periods of 45 days were recorded by the isolates TLF-8, CLF-13 and CCB-8 on ayurvedic waste,

**Table 16. *In vivo* screening of microbial degraders on respective host substrates**

| Sl.No. | Microbial degraders | *Days for maturity | Host substrate |
|---|---|---|---|
| 1 | ACF-6 | 50 | Ayurvedic waste |
| 2 | ALF-26 | 45 | Ayurvedic waste |
| 3 | ACB-2 | 50 | Ayurvedic waste |
| 4 | ACA-5 | 50 | Ayurvedic waste |
| 5 | ALA-1 | 50 | Ayurvedic waste |
| 6 | ATY-1 | 50 | Ayurvedic waste |
| 7 | CLF-6 | 55 | Coir pith |
| 8 | CCF-4 | 50 | Coir pith |
| 9 | CLF-12 | 50 | Coir pith |
| 10 | CLF-13 | 45 | Coir pith |
| 11 | CLF-16 | 50 | Coir pith |
| 12 | CLF-22 | 55 | Coir pith |
| 13 | CCB-8 | 50 | Coir pith |
| 14 | ECF-2 | 50 | Elephant dung |
| 15 | ECA-8 | 50 | Elephant dung |
| 16 | CaLF-5 | 65 | Cashew leaves |
| 17 | CaLF-7 | 70 | Cashew leaves |
| 18 | CaTF-7 | 70 | Cashew leaves |
| 19 | CaCA-3 | 70 | Cashew leaves |
| 20 | CaCA-5 | 70 | Cashew leaves |
| 21 | TLF-8 | 60 | Teak leaves |
| 22 | CdCB-1 | 55 | Paddy straw |

*Mean of two replications

## PLATE 4

### SCREENING OF SELECTED DEGRADERS ON HOST SUBSTRATES

### A. *In vitro*

Conical flask

Plastic pot

### B. *In Vivo*


Ayurvedic waste


Coir pith


Leaf litters


Paddy straw

**Table 17. *In vivo* screening of microbial degraders on other host substrates**

| Sl.No. | Microbial degraders | *Days for maturity | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Ayurvedic waste | Coir pith | Elephant dung | Teak leaves | Mango leaves | Cashew leaves | Ordinary plant waste |
| 1 | ACF-6 | 50 | 55 | 50 | 65 | 70 | 70 | 55 |
| 2 | ALF-26 | 45 | 50 | 50 | 65 | 70 | 70 | 50 |
| 3 | ACB-2 | 50 | 55 | 50 | 65 | 70 | 70 | 55 |
| 4 | ACA-5 | 50 | 50 | 50 | 65 | 70 | 70 | 55 |
| 5 | ALA-1 | 50 | 55 | 50 | 65 | 70 | 70 | 55 |
| 6 | ATY-1 | 50 | 45 | 50 | 60 | 70 | 70 | 55 |
| 7 | CLF-6 | 50 | 55 | 50 | 65 | 70 | 70 | 55 |
| 8 | CCF-4 | 50 | 50 | 50 | 65 | 70 | 70 | 55 |
| 9 | CLF-12 | 45 | 50 | 50 | 65 | 70 | 70 | 55 |
| 10 | CLF-13 | 50 | 45 | 50 | 60 | 65 | 70 | 50 |
| 11 | CLF-16 | 50 | 50 | 50 | 65 | 70 | 70 | 55 |
| 12 | CLF-22 | 50 | 55 | 50 | 65 | 70 | 70 | 55 |
| 13 | CCB-8 | 45 | 50 | 45 | 65 | 70 | 70 | 55 |
| 14 | ECF-2 | 50 | 50 | 50 | 60 | 70 | 70 | 55 |
| 15 | ECA-8 | 50 | 50 | 50 | 65 | 65 | 65 | 55 |
| 16 | CaLF-5 | 50 | 45 | 45 | 60 | 65 | 65 | 55 |
| 17 | CaLF-7 | 50 | 50 | 45 | 65 | 70 | 70 | 55 |
| 18 | CaTF-7 | 50 | 50 | 50 | 65 | 70 | 70 | 55 |
| 19 | CaCA-3 | 50 | 55 | 50 | 65 | 70 | 70 | 55 |
| 20 | CaCA-5 | 50 | 50 | 50 | 65 | 70 | 70 | 55 |
| 21 | TLF-8 | 45 | 45 | 45 | 60 | 70 | 70 | 55 |
| 22 | CdCB-1 | 50 | 50 | 50 | 65 | 70 | 70 | 55 |

*Mean of two replications

ATY-1, CaLF-5, TLF-8 on coir pith, CaLF-5 and 7, TLF-8 on elephant dung, 60 days by ATY-1and CLF-13 on teak and mango leaves and 65 days by ECA-8 and CaLF-5 on cashew leaves. This result indicated the capability of these organisms to degrade any lignin- tannin rich agrowastes.



## 4.8. STUDIES ON MUTUAL COMPATIBILITY BETWEEN THE SELECTED MICROBIAL DEGRADERS

Degraders selected from *in vivo* experiment were tested for mutual compatibility which is essential for the development of consortium and the interaction between the various organisms are detailed in Table 18.

### 4.8.1. Compatibilty between selected fungal degraders

Thirty six combinations of nine fungi were observed for their mutual interactions and the results revealed that, all degraders were mutually compatible as no lysis or overgrowth or inhibition was observed among the combinations (Plate 5 A).

Of the 36 combinations fungi mainly exhibited independent growth without intermingling and over growth. Six fungal combinations showed hyphal intermingling at the meeting point. Some isolates showed thickening of hyphae at interaction point. Five combinations with CLF-13 and CLF-12 showed yellowish brown metabolite on the reverse side of the medium without diffusion to the site of other one. Disintegration of cells or overgrowth among the isolates was not observed.

### 4.8.2. Compatibiltiy between fungi and bacteria or actinomycetes or yeast

The tested nine fungi did not show any clear zones on the bacteria/ actinomycete/ yeast seeded PDA media indicating their compatibility to each other (Plate 5 B).

**Table 18. Mutual compatibility between selected fungal degraders**

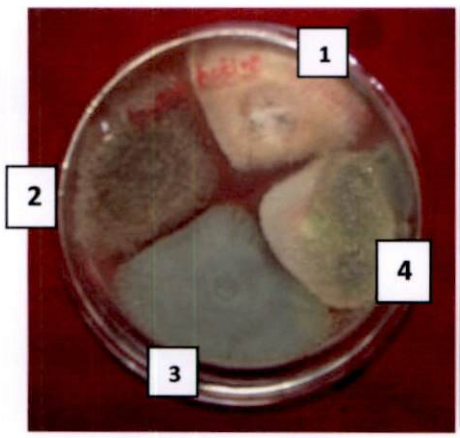
| Sl.No | Microbial degraders | Observations |
|---|---|---|
| 1 | ECF-2 x ALF-26 | Intermingling of hyphae at the meeting point |
| 2 | ECF-2 x CaLF-5 | No inhibition/ lysis, no overgrowth |
| 3 | ECF-2 x TLF-8 | Intermingling and thickening of hyphae at the interaction point. No over growth, no disintegration of cells. |
| 4 | ECF-2 x CaTF-7 | |
| 5 | ECF-2 x CLF-12 | Intermingling of hyphae at the contact point. No over growth. |
| 6 | ECF-2 x CLF-16 | Thickening of hyphae at the contact point. No over growth. |
| 7 | ECF-2 x CLF-6 | |
| 8 | ECF-2 x CCF-4 | Intermingling and thickening of hyphae at the interaction site |
| 9 | ALF-26 x CaLF-5 | No lysis / inhibition. Metabolite production on reverse side of the colony of CaLF-5, but no diffusion toALF-26. No overgrowth. |
| 10 | ALF-26 x TLF-8 | No intermingling, no overgrowth |
| 11 | ALF-26 x CaTF-7 | |
| 12 | ALF-26 x CLF-12 | Intermingling of hyphae at the meeting point. Metabolite production on reverse side of CLF -12 and colour diffuses. |
| 13 | ALF-26 x CLF-16,CLF-6 | No intermingling and over growth |
| 14 | ALF-26 x CCF-4 | Intermingling of hyphae at the meeting point. No over growth |
| 15 | CaLF-5 x TLF-8 | No overgrowth. No lysis |
| 16 | CaLF-5 x CaTF-7 | |
| 17 | CaLF-5 x CLF-12 | No over growth. No lysis |
| 18 | CaLF-5 x CLF-16 | No intermingling. No over growth. Metabolite production on the reverse side of CaLF-5 but not diffused to other one. |
| 19 | CaLF-5 x CLF-6 | No over growth. Independent growth. Metabolite production on reverse side of CaLF-5, but no diffusion. |
| 20 | CaLF-5 x CCF-4 | No over growth. No lysis of cells |

| | | |
|---|---|---|
| 21 | TLF-8 x CaTF-7 | No lysis of cells. No over growth |
| 22 | TLF -8 x CLF-12 | No intermingling. No over growth. Metabolite production on reverse side of CLF-12, but no diffusion. |
| 23 | TLF-8 x CLF-16,CLF-6 & CCF-4 | No lysis of cells. No over growth |
| 24 | CaTF-7 x CLF-12, CLF-16, CLF-6 & CCF-4 | |
| 25 | CLF-12 x CLF16,CLF-6 & CCF-4 | |
| 26 | CLF-16 x CLF-6 & CCF-4 | |
| 27 | CLF-6 x CCF-4 | |

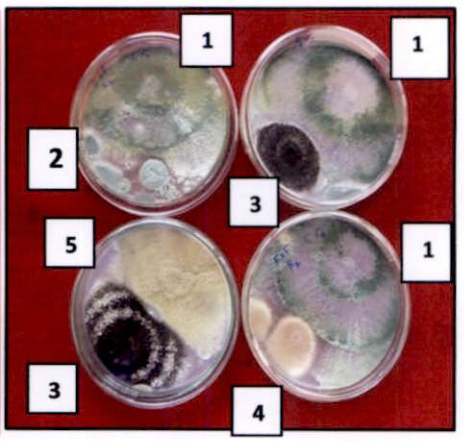
# PLATE 5

## MUTUAL COMPATIBILITY OF SELECTED MICROBIAL DEGRADERS

### A. Fungi x Fungi

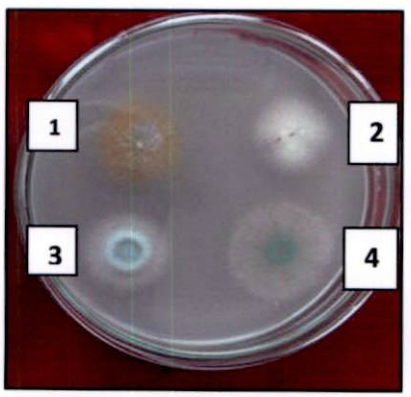



**1.** ECF-2 **2.** ALF-26 **3.** CaTF-7. **4.** CaLF-5



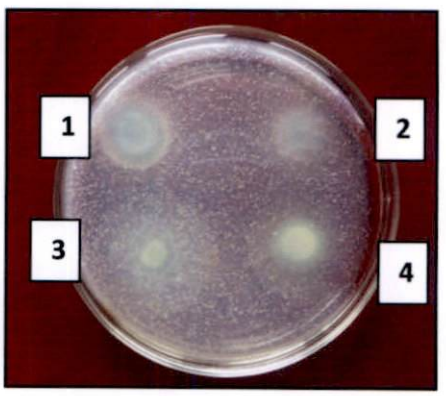

**1.** ECF-2 **2.**CaLF-5 **3.** CaLF-7 **4.** TLF- 8 **5.** ALF-26

### B. Bacteria x Fungi



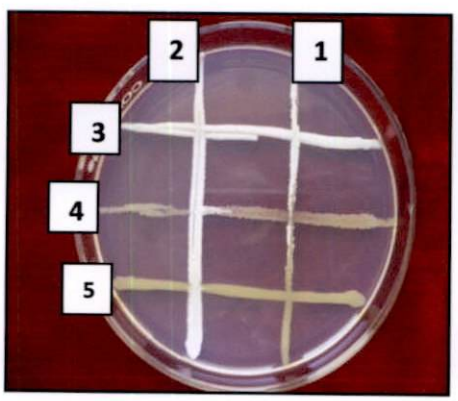

**1.**ALF-26 **2.** TLF-8 **3.**CaLF-5 **4.** ECF-2

### B. Acitnomycete x Fungi



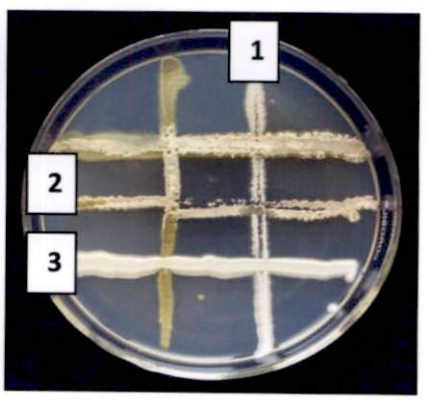

**1.** ECF-2 **2.** ALF-26 **3.**CaLF-5 **4.**CaTF-7

### C. Bacteria x Actinomycete




**1.** CdCB-1 **2.** CCB-8 **3.** CaCA-3 **4.**ACA-5 **5.**ALA-1

### C. Actinomycete x Actinomycete




**1.** CaCA-3 **2.** ACA -5 **3.**ALA- 1

### 4.8.3. Compatibilty between bacteria and bacteria or actinomycetes or yeast

Bacterial degraders were tested against actinomycetes and yeast by cross streaking method and none of the degraders showed lysis at the junctures, indicating their mutual compatibility (Plate 5 C).

## 4.9. IDENTIFICATION OF SELECTED MICROBIAL DEGRADERS

Fungi, bacteria, actinomycetes and yeast selected for microbial consortium were identified based on cultural, morphological, biochemical and molecular characters.

### 4.9.1. Identification of fungal degraders

Twenty two fungal degraders tested *in vivo* were identified to species level and presented in Table 19.

The cultural and morphological characters of five fungal degraders selected for microbial consortium are detailed below.

1. **Isolate No.ECF-2**

   **Cultural characters**

   Colonies : Fast growth, white green to dull green

   **Morphological characters**

   Mycelium : Hyaline, septate, smooth

   Coniodiophore : Loose tuft, main branches of conidiophores produced numerous side branches specially over lower portion

   Phialides: : Arise in false verticils upto five which are short skittle shaped, narrow at the base, attenuated abruptly with sharp pointed neck, 7.1 x 2.7 μm in size

   Conidia : Accumulated at the tip of phialides, subglobose, short obovoid, often with broad, truncate base, smooth, pale green, much darker in mass, 2.3 x 1.8μm in size.

## Table 19. Identification of selected microbial degraders

| Sl. No. | Isolate No. | Identification No. | Name of identified isolate | Type of Organism | Substrate | Degrader |
|---|---|---|---|---|---|---|
| 1 | ACF-6 | NFCCI-1.2 | *Aspergillus fumigatus* | Fungus | Ayurvedic | Cellulose |
| 2 | ALF-26 | NCFT - 5374.12 | *Paecilomyces variotii* | Fungus | Ayurvedic | Lignin |
| 3 | CCF-4 | NFCCI - 3.2 | *Fusarium fusarioides* | Fungus | Coir pith | Cellulose |
| 4 | CLF-6 | NCFT-5857.14 | *Penicllium chrysogenum* | Fungus | Coir pith | Lignin |
| 5 | CLF-12 | NFCCI - 5.1 | *Aspergillus nidulans* | Fungus | Coir pith | Lignin |
| 6 | CLF-13 | NFCCI - 2.1 | *Penicillium citrinum* | Fungus | Coir pith | Lignin |
| 7 | CLF-16 | NFCCI - 8.2 | *Penicillium chrysogenum* | Fungus | Coir pith | Lignin |
| 8 | CLF-22 | NFCCI -5.2 | *Aspergillus terreus* | Fungus | Coir pith | Lignin |
| 9 | ECF-2 | NFCCI - 4.1 | *Trichoderma harzianum* | Fungus | Elephant dung | Cellulose |
| 10 | CaLF-5 | NCFT-5858.14 | *Penicillium oxalicum* | Fungus | Cashew | Lignin |
| 11 | CaLF-7 | NCFT-5377.12 | *Aspergillus sclerotioum* | Fungus | Cashew | Lignin |
| 12 | CaTF-7 | NCFT-6135.14 | *Penicillium chrysogenum* | Fungus | Cashew | Tannin |
| 13 | TLF-8 | NFCCI -6.2 | *Talaromyces* sp. | Fungus | Teak | Lignin |
| 14 | ACB-2 | D-6 | *Achromobacter xylosoxidans* | Bacterium | Ayurvedic | Cellulose |
| 15 | CCB-8 | D-5 | *Massilia* sp. | Bacterium | Coir pith | Cellulose |
| 16 | CdCB-1 | D-3 | *Athrobacter* sp. | Bacterium | Cow dung | Cellulose |
| 17 | ACA-5 | NCFT-5369.12 | *Streptomyces niveus* | Actinomycete | Ayurvedic | Cellulose |
| 18 | ALA-1 | NCFT-5372.12 | *Streptomyces albus* | Actinomycete | Ayurvedic | Lignin |
| 19 | ECA-8 | NCFT-5859.14 | *Streptomyces albus* | Actinomycete | Elephant dung | Cellulose |
| 20 | CaCA-3 | NCFT-5860.14 | *Streptomyces albus* | Actinomycete | Cashew | Cellulose |
| 21 | CaCA-5 | NCFT-5862.14 | *Streptomyces albus* | Actinomycete | Cashew | Cellulose |
| 22 | ATY-1 | NCFT-5861.14 | *Candida* sp. | Yeast | Ayurvedic | Tannin |

Based on the above characters, the isolate was tentatively identified as *Trichoderma harzianum* and confirmed from National Fungal Culture Collection of India (NFCCI), Pune IDNo.- 4.1(Plate 6 A).

**2. Isolate No. ALF-26**

**Cultural characters**

Colonies : Fast growth, powdery to suede – like and tufted. Colour varied from yellowish brown or sand colour.

**Morphological characters**

Mycelium : Septate, hyaline

Coniodiophores :Loosely branched and irregularly brush like and size 15.9 x 2.2 µm

Phialides: : Swollen at the base which gradually tapered to a sharp point at the tip and 2.1 x 2.5µm in size

Conidia : Dark coloured, smooth or rough, oval to fusoid, and form long chains.

Based on the above characters, the isolate was tentatively identified as *Paecilomyces variotii* and confirmed from National Centre for Fungal Taxonomy (NCFT), New Delhi, ID No. - 5372.12 (Plate 6 B).

**3. Isolate No. CaLF-5**

**Cultural characters**

Colonies : Plane to radially sulcate, velutinous to floccose, blue green in colour with pale yellow pigmentation on the reverse side

**Morphological characters**

Mycelium : Septate

Coniodiophores : Long penicillin, biverticillate with 2-3 metulae closely appressed or mono verticillate

Phialides: : Cylindrical to acerose with size 9.5 x 3.3µm

Conidia : Ellipsoidal, having smooth walls borne in long columns of 3.5 x 2.8 µm

Based on above characters the isolate was tentatively identified as *Penicillium oxalicum* and confirmed from NCFT, New Delhi, ID No.-5858.14 (Plate 6C).

4. **Isolate No. TLF-8**

**Cultural characters**

Colonies : Moderately floccose with bright yellow mycelium. The reverse side of the medium appeared reddish.

**Morphological characters**

Mycelium : Septate

Ascospores : Ellipsoidal stripes bearing and terminal, commonly biverticillate

Ascoma : Yellow gymnothecia.

Based on the above characters, the isolate was tentatively identified as *Talaromyces* sp. and confirmed from NFCCI, Pune, ID No. - 6.2 (Plate 6 D).

5. **Isolate No. CaTF-7**

**Cultural characters**

Colonies : Radially sulcate, velutinous to floccose, greyish blue green in colour

**Morphological characters**

Mycelium : Branched, septate

Coniodiophores : Smooth walled, terminating in terverticillate penicillin with 1- 2 rami

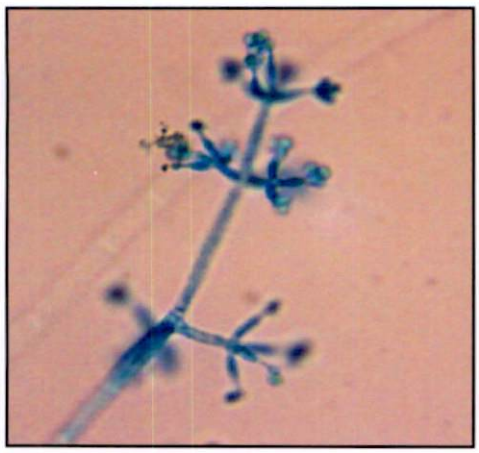
Phialides: : Ampulliform, 4 -7 per metulae and size- 14.18 x2.88 µm

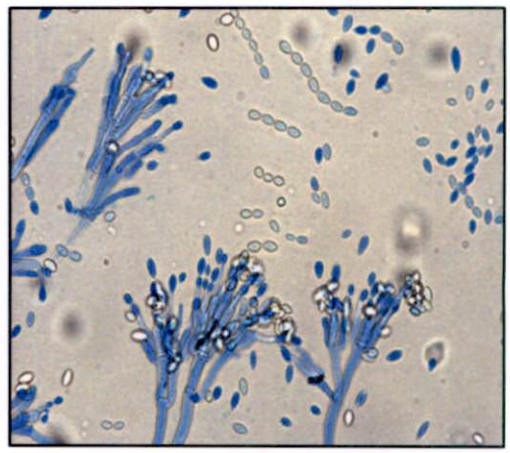
Conidia : Ellipsoidal to subspheroidal, smooth walled, borne in long irregular columns and 2.40 x 2.00 µm in size

Based on the above characters, the isolate was tentatively identified as *Penicillium chrysogenum* and confirmed from NCFT, New Delhi, ID No. - 5858.14 (Plate 6E).

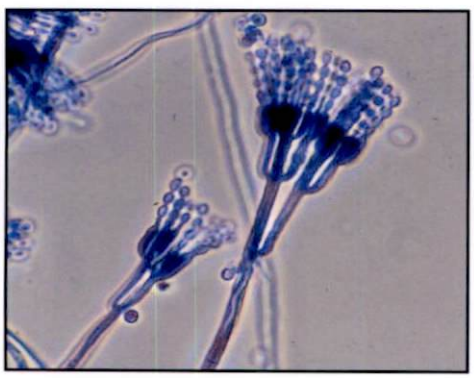
# PLATE 6

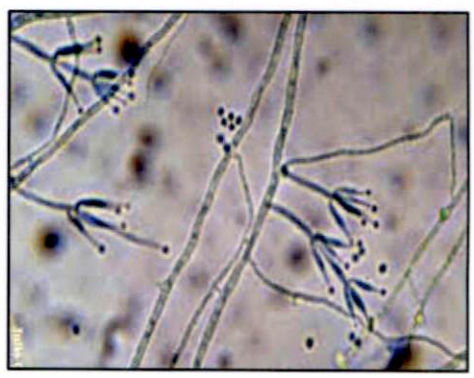
## IDENTIFICATION OF SELECTED FUNGAL DEGRADERS



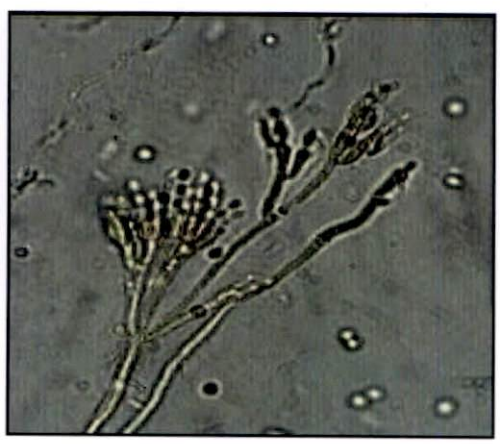


A.*Trichoderma harzianum* (10 x 40 X) B. *Paceilomyces variotii* (10 x100 X)





C. *Penicillium oxalicum* (10 x 40 X) D. *Talaromyces* sp. (10 x 40 X)



E. *Penicillium chrysogenum* (10 x 100 X)

### 4.9.2. Identification of bacterial degraders

Selected two bacteria were identified based on cultural, morphological, biochemical and molecular characters and are presented below.

**1. Isolate No. CdCB-1**

**Cultural characters** : Colonies are circular, convex, fluidal, and yellow with entire margins

**Morphological characters** : Gram positive, short rods

**Biocehmical characterisation**

The isolate showed positive reaction to catalase, denitrification, nitrate reduction and glucose and arabinose utilization (Table 20).

**Molecular characterisation**

The sequence analysis of the isolate CdCB-1 showed 97 per cent query coverage and 89 per cent identity to *Arthrobacter* sp. (NCBI accession No.JX 290308.1) (Table 21).

Based on the cultural morphological, biochemical characters and by 16S rRNA sequencing the bacterium was identified as *Arthrobacter* sp.

**2. Isolate No. CCB-8**

**Cultural characters** : The colonies were convex, straw coloured and dry with irregular margins

**Morphological characters:** Gram negative, short rods

**Biocehmical characterisation**

The isolate recorded positive reaction for catalase, starch hydrolysis, gelatine liquefaction, reduction of nitrate and negative reaction to sugar utilization (Table 20).

**Table 20. Cultural, morphological and biochemical characters of selected bacterial degraders**

| Sl.No. | Test | CdCB-1 | CCB-8 |
|---|---|---|---|
| 1 | Margin | Irregular | Entire |
| 2 | Elevation | Convex | Convex |
| 3 | Colour | Yellow | Straw |
| 4 | Texture | Fluidal | Dry |
| 5 | Gram's staining | + | - |
| 6 | Shape | rod | rod |
| 7 | Catalase test | + | + |
| 8 | Denitrification | + | - |
| 9 | Starch hydrolysis | - | + |
| 10 | Gelatin liquefaction | - | + |
| 11 | Citrate utilization | - | - |
| 12 | Lysine utilization | - | - |
| 13 | Ornithine utilization | - | - |
| 14 | Urease detection | - | - |
| 15 | Phenylalanine deamination | - | - |
| 16 | Nitrate reduction | + | + |
| 17 | Hydrogen sulphide production | - | - |
| 18 | **Utilization of sugars & alcohols** Glucose | + | - |
| 19 | Sucrose | - | - |
| 20 | Lactose | - | - |
| 21 | Arabinose | + | - |
| 22 | Adonitol | - | - |
| 23 | Sorbitol | - | - |

\+ Positive -- Negative

**Table 21. Molecular characterisation of bacterial degraders**

| Isolate No. | NCBI accession No | Description | Max. score (%) | e-value | Query coverage (%) | Identities (%) |
|---|---|---|---|---|---|---|
| CdCB-1 | JX290308.1 | *Arthrobacter* sp. | 1563 | 0.0 | 97 | 89 |
| | HM123757.1 | *Arthrobacter* sp. | 1563 | 0.0 | 97 | 89 |
| | KF054859.1 | *Arthrobacter mysorens* | 1563 | 0.0 | 97 | 89 |
| | AB210984.1 | *Arthrobacter protophormiae* | 1563 | 0.0 | 97 | 89 |
| | HM480363.1 | *Arthrobacter nicotianae* | 1561 | 0.0 | 94 | 89 |
| CCB-8 | AB552860.1 | *Massilia* sp. | 96 | 0.0 | 96 | 92 |
| | EU274637.2 | *Massilia timonae* | 96 | 0.0 | 96 | 92 |
| | KF10673.1 | Uncultured bacteria | 96 | 0.0 | 96 | 91 |
| | HQ120383.1 | Uncultured bacteria | 96 | 0.0 | 96 | 91 |
| | EU431741.1 | Uncultured bacteria | 96 | 0.0 | 96 | 91 |

**Molecular characterisation**

The sequence analysis of the isolate, CCB-8 recorded 96 per cent query coverage and 92 per cent identity to *Massilia* sp. (NCBI Accession No. AB 552860.1 (Table 21).

Based on the cultural, morphological, biochemical characters and by 16srRNA sequencing, the bacterium was identified as *Massilia* sp.

**4.9.3. Identification of actinomycetes degraders**

The morphological and cultural characters of the actinomycetes were studied based on the standard keys and the results are presented in Table 22.

1. **Isolate No. ACA- 5**

Colony size of the isolate was medium. Both substrate and aerial mycelium were present. Fragmentation of substrate mycelium and motility of spores were absent. The colonies of the isolate later covered with aerial mycelium bearing chains of arthrospores. Colour of the spore mass was grey. Production of pigment was not noticed on the reverse side. Based on the cultural characters, ACA-5 was tentatively identified as *Streptomyces* sp. and was identified as *S. niveus* from NCFT, New Delhi, ID No. - 5 369.12.

2. **Isolate No. ALA- 1 and CaCA -3**

The colony size of the isolate was medium. Both substrate and aerial mycelia were present. Fragmentation of substrate mycelium and motility of spores were absent and the colonies of the isolate were later covered with aerial mycelium bearing chains of arthrospores. Colour of the spore mass was white. Production of pigment was not noticed on the reverse side of both isolates. Based on the cultural characters, ALA-1 and CaCA-3 were tentatively identified as *Streptomyces* sp. and were identified as *S. albus* from NCFT, New Delhi, ID No. -5372.12 and ID No. – 5860.14.

## Table 22. Cultural characters of actinomycete degraders

| Sl . No. | Characters | ACA-5 | CaCA-3 | ALA -1 |
|---|---|---|---|---|
| 1 | Colony size | Discrete | Discrete | Discrete |
| 2 | Substrate mycelium | + | + | + |
| 3 | Chain of spores | - | - | - |
| 4 | Motile spores | - | - | - |
| 5 | Fragmentation of substrate mycelium | - | - | - |
| 6 | Aerial mycelium | + | + | + |
| 7 | Chain of arthrospores | + | + | + |
| 8 | Arthrospores in verticils | - | - | - |
| 9 | Spore chain morphology | Spirales | Spirales | Spirales |
| 10 | Spore surface ornamentation | Smooth | Smooth | Smooth |
| 11 | Colour of spore mass | Grey | Grey | Grey |
| 12 | Pigmentation of substrate mycelium | yellow | pink | White |
| 13 | Diffusible pigments | - | - | - |
| 14 | Probable genus | *Streptomyces* | *Streptomyces* | *Streptomyces* |

\+ Positive -- Negative

### 4.9.4. Identification of yeast degrader

Yeast selected for consortium was identified tentatively based on colony morphology. The colonies in chains were small, raised, creamy white with smooth texture. This was identified as *Candida* sp. from NCFT, New Delhi, ID No. - 5861.14.

## 4.10. DEVELOPMENT OF MICROBIAL CONSORTIUM

Based on the ability to degrade all the three chemical components, type and species of organisms, early maturity period of composting under *in vitro* and *in vivo* studies and mutual compatibility, 13 efficient degraders were selected for the formulation of microbial consortium (Table 23). Microbial consortia were developed with and without *Aspergillus* spp.

Consortium –I, was developed with 10 degraders representing all the substrates and chemical components. As the *in vitro* studies showed *Aspergillus* spp. as the most efficient degraders of all the three chemical components, two non human pathogenic *Aspergillus* spp. were included in this consortium, which consisted of *Trichoderma harzianum* (ECF-2), *Paecilomyces variotii* (ALF-26), *Penicillium citrinum* (CLF-13), *Aspergillus nidulans* (CLF-12), *A. sclerotiorum* (CaLF-7), *Arthrobacter* sp. (CdCB-1), *Massilia* sp. (CCB-8), *Streptomyces niveus* (ACA-5), *S. albus* (ALA-1) and *Candida* sp.(ATY-1).

Consortium (II) with 10 degraders included *T. harzianum* (ECF-2), *P. variotii* (ALF-26), *Penicillium oxalicum* (CaLF-5), *P. chrysogenum* (CaTF-7), *Talaromyces* sp. (TLF-8), *Arthrobacter* sp. (CdCB-1), *Massilia* sp. (CCB-8), *S. niveus* (ACA-5), *S. albus* (ALA-1) and *S. albus* (CaCA-3).

**Table 23. Degraders selected for development of microbial consortium**

| Sl.No. | Isolate No. | Name of isolate | Base substrate | Best degraded substrates | Component degraded |
|---|---|---|---|---|---|
| 1 | ECF -2 | *Trichoderma harzianum* | Elephant dung | Ayurvedic waste, Coir pith, Elephant dung | Cellulose |
| 2 | ALF-26 | *Paecilomyces variotii* | Ayurvedic waste | Ayurvedic waste, Coir pith, Elephant dung | Lignin |
| 3 | CLF-13 | *Penicillium citrinum* | Coir pith | Ayurvedic waste, Coir ptih, Elephant dung, Leaf litters | Lignin |
| 4 | CaLF-5 | *Penicillium oxalicum* | Cashew leaves | Ayurvedic waste, Coir ptih, Elephant dung, Leaf litters | Lignin |
| 5 | TLF-8 | *Talaromyces* sp. | Teak leaves | Ayurvedic waste, Coir pith, Elephant dung | Lignin |
| 6 | CaTF-7 | *Penicillium chrysogenum.* | Cashew leaves | Ayurvedic waste, Coir pith, Elephant dung | Tannin |
| 7 | CdCB-1 | *Arthrobacter* sp. | Cow dung | Ayurvedic waste, Coir pith, Elephant dung | Cellulose |
| 8 | CCB-8 | *Massilia* sp. | Coconut by products | Ayurvedic waste, Coir pith, Elephant dung | Cellulose |
| 9 | ACA-5 | *Streptomyces niveus* | Ayurvedic waste | Ayurvedic waste, Coir pith, Elephant dung | Cellulose |
| 10 | ALA-1 | *Streptomyces albus* | Ayurvedic Waste | Ayurvedic waste, Coir pith, Elephant dung | Lignin |
| 11 | CaCA-3 | *Streptomyces albus* | Cashew leaves | Ayurvedic waste, Elephant dung, Mango leaves | Cellulose |
| 12 | ATY-1 | *Candida* sp. | Ayurvedic Waste | Ayurvedic waste, Coir pith, | Tannin |
| 13 | CLF-12 | *Aspergillus nidulans* | Coir pith | Ayurvedic waste, Coir pith,Elephant dung | Lignin |
| 14 | CaLF-7 | *Aspergillus sclerotiorum* | Cashew leaves | Ayurvedic waste, Elephant dung, Coir pith | Lignin |

## 4.11. *IN VITRO* SCREENING OF MICROBIAL CONSORTIUM

Two microbial consortia developed were tested for their efficacy to degrade selected ligno-phenolic substrates under *in vitro* conditions along with normal composting with 20 per cent cow dung as control (Table 24).

The substrates treated with microbial consortium –II recorded early maturity as compared to those treated with microbial consortium – I (with *Aspergillus* spp.) and normal composting with cow dung slurry. Among the substrates, mixed substrates showed lowest maturity period irrespective of treatments.

Substrates treated with microbial consortium –II recorded lowest maturity periods varied from 35 - 57 days, of which, mixed substrate and coir pith showed minimum maturity periods of 35 days followed by ayurvedic waste with 45 days, whereas, in case of microbial consortium –I, maturity period ranged from 45 - 60 days and same trend was observed with respect to the composting period of various substrates.

The normal composting with cow dung slurry showed slightly longer maturity periods as compared to consortium treated ones in which maturity period varied from 65 - 80 days, of which, mixed substrate showed early maturity.

In all treatments, leaf litters recorded comparatively longer maturity periods ranged from 57 - 80 days with minimum in consortium –II treatment and microbial consortium –II was selected for further studies.

**Table 24. *In vitro* screening of microbial consortium on different substrates**

| Sl. No. | Substrates | *Maturity period (days) | | | |
|---|---|---|---|---|---|
| | | Consortium I | Consortium II | Cow dung slurry | Control |
| 1 | Ayurvedic waste | 50 | 45 | 75 | 100 |
| 2 | Coir pith | 45 | 35 | 70 | 95 |
| 3 | Leaf litters | 60 | 57 | 80 | 105 |
| 4 | Mixed substrates | 45 | 35 | 65 | 85 |

*Mean of three replications

## 4.12. *IN VIVO* SCREENING OF MICROBIAL CONSORTIUM

An *in vivo* experiment was conducted to evaluate the efficacy of the selected microbial consortium along with normal composting with cow dung slurry (20 %) as check. The results furnished in Table 25, showed a similar trend as that observed in *in vitro* study and the substrates treated with the microbial consortium recorded early maturity compared to that treated with cow dung slurry.

The maturity periods of different substrates varied from 43 – 65 days with consortium and 50 -72 days with cow dung slurry applications. In both treatments, mixed substrate showed lowest maturity period of 43 and 50 days respectively. The microbial consortium treated coir pith and ayurvedic waste recorded maturity periods of 45 and 49 days against 53 and 56 days in cow dung treated one.

As observed in the *in vitro* experiments, leaf litters showed longest composting periods (65 and 72 days) in both consortium and cow dung applications.

## 4.13. LARGE SCALE COMPOSTING OF LIGNO-PHENOLIC SUBSTRATES WITH MICROBIAL CONSORTIUM

Effect of microbial consortium was tested again with large quantity of substrates in concrete tanks along with treatments of cow dung slurry alone, combination of consortium and cow dung slurry and uninoculated control (Plate 7) the compost maturity was assessed based on temperature, texture and C:N ratio and the experimental results are presented in Table 26.

Among the treatments, application of 20 per cent microbial consortium alone showed early maturity of all substrates and lowest composting period (30 days) was observed in mixed substrate followed by 35 in coir pith, 40 in ayurvedic waste and 55 days in leaf litters.

**Table 25. *In vivo* screening of microbial consortium on different substrates**

| Sl. No. | Substrates | *Maturity period (days) | | |
|---|---|---|---|---|
| | | Microbial consortium (20 %) | Cow dung slurry (20 %) | Control |
| 1 | Ayurvedic waste | 49 | 56 | 76 |
| 2 | Coir pith | 45 | 53 | 75 |
| 3 | Leaf litters | 65 | 72 | 95 |
| 4 | Mixed substrates | 43 | 50 | 70 |

*Mean of three replications

**Table 26. Large scale composting of various substrates with liquid formulation of microbial consortium**

| Sl. No. | Substrates | * Maturity period (days) | | | |
|---|---|---|---|---|---|
| | | Consortium alone (20 %) | Cow dung slurry alone (20%) | Consortium + cow dung slurry (20 %) | Control |
| 1 | Ayurvedic waste | 40 | 50 | 56 | 96 |
| 2 | Coir pith | 35 | 45 | 53 | 80 |
| 3 | Leaf litter | 55 | 65 | 66 | 130 |
| 4 | Mixed substrates | 30 | 42 | 35 | 82 |
| 5 | Normal composting with ordinary crop residues (check) | - | 46 | - | - |

*Mean of three replications

# PLATE 7

## LARGE SCALE COMPOSTING OF VARIOUS SUBSTRATES WITH MICROBIAL CONSORTIUM


General view of composting

Consortium treated coir pith

## MATURE COMPOSTS

Ayurvedic

Coir pith

Leaf litters

Mixture

The maturity periods were found to be prolonged in treatments with cow dung slurry alone as well as in combination with microbial consortium and in both cases, lowest periods were observed in mixed substrates. Composting periods of 42, 45, 50 and 65 days were noted for cow dung slurry alone and 35, 53, 56 and 66 days in combination treatments with respect to mixed substrates, coir pith, ayurvedic waste and leaf litters. The normal composting of ordinary cellulose rich plant residues with 20 per cent cow dung slurry recorded 46 days for maturity.

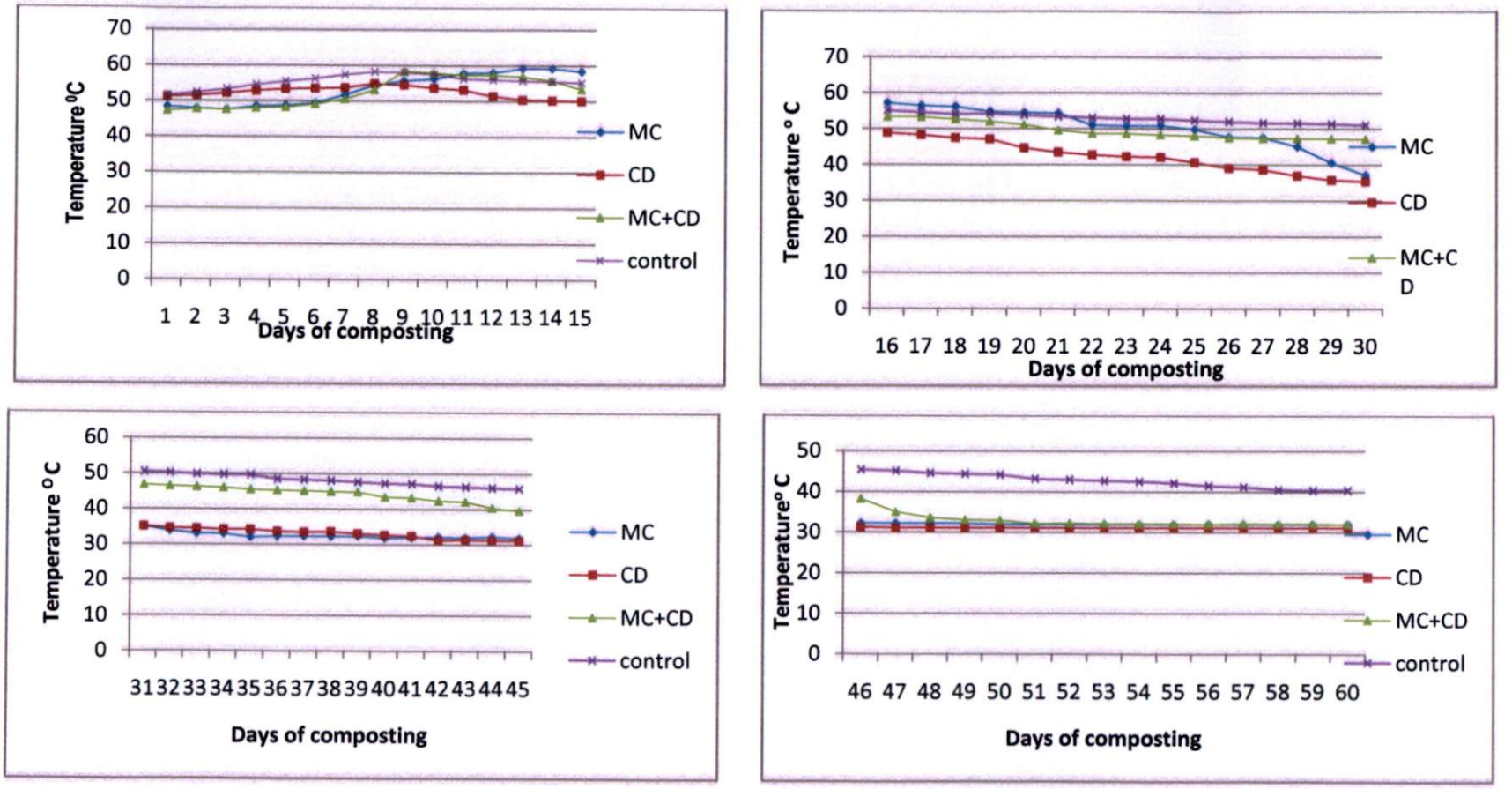
### 4.13.1. Variation in temperature during composting

Daily temperature variation during composting were recorded and presented in Fig 1- 4. Irrespective of the treatments, temperature of the composting piles showed a steady increase to a maximum value which coincided with the thermophilic phase and thereafter gradually decreased to minimum at compost maturity.

Among the substrates, high initial temperature of 37.6 - 48.4 °C was recorded in ayurvedic waste which further increased to peak value of 59.2°C on 13$^{th}$ day of composting with microbial consortium followed by that treated with combination of cow dung slurry and microbial consortium (56.1°C). The temperature peaks of various treatments later steadily declined to 30.2 - 31.0 °C at maturity.

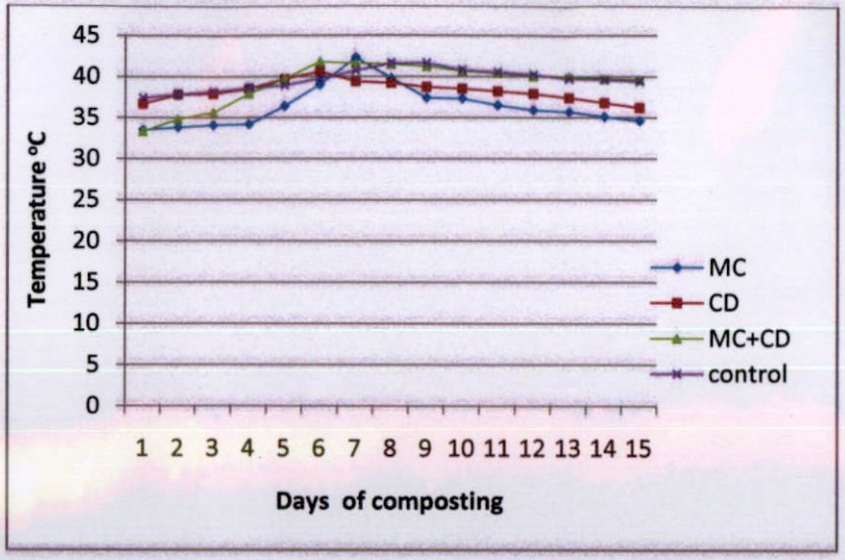
Coir pith showed initial temperature range of 33.3 - 37.4°C in various treatments. Peak temperature of 42.3°C was observed in the treatment with microbial consortium on 7$^{th}$ day followed by combination of cow dung slurry and microbial consortium (41.8°C). The temperature later decreased at maturity and ranged from 32.1- 34.6°C in different treatments.

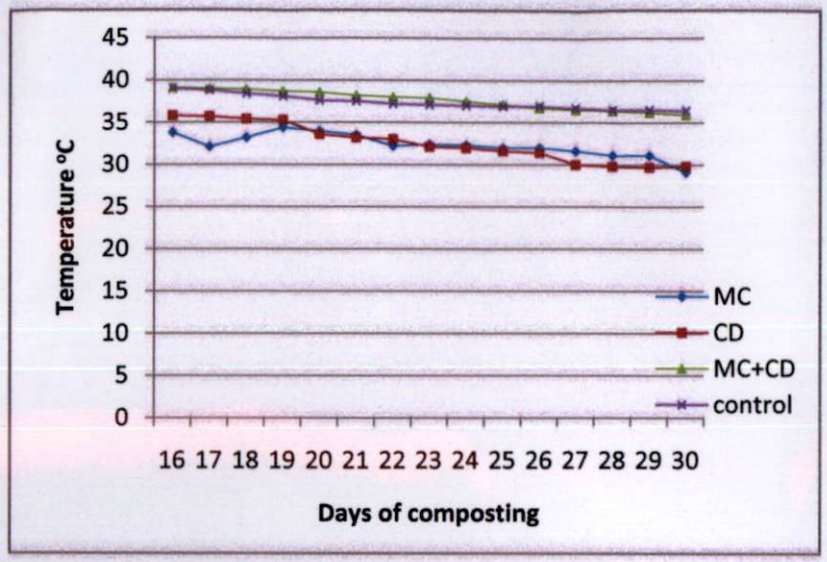
Leaf litters recorded lowest temperatures among various substrates throughout the composting period with initial temperatures of 32.1- 34.6° C. The temperature showed steady increase to maximum of 35.7°C on 6$^{th}$ day of

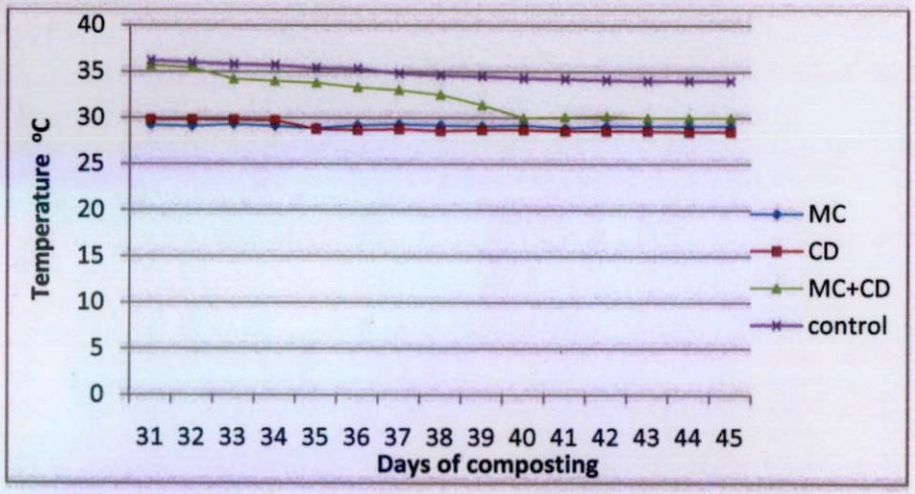



MC –Microbial consortium, CD – Cow dung, MC + CD - Microbial consortium + Cow dung

**Fig 1. Temperature variations during composting of ayurvedic waste**

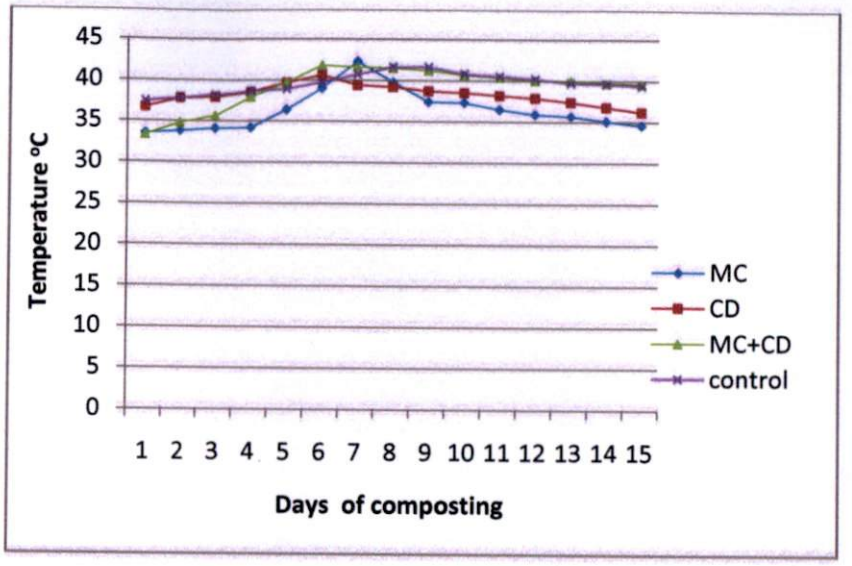


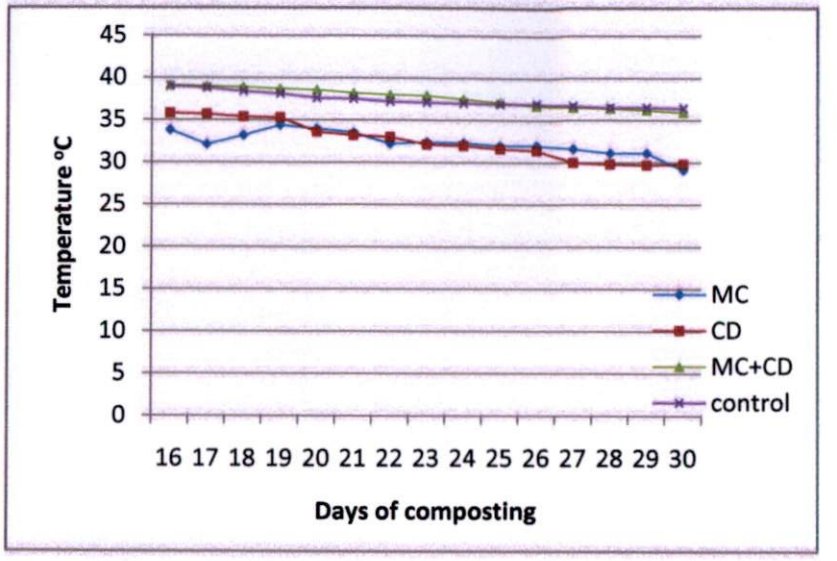



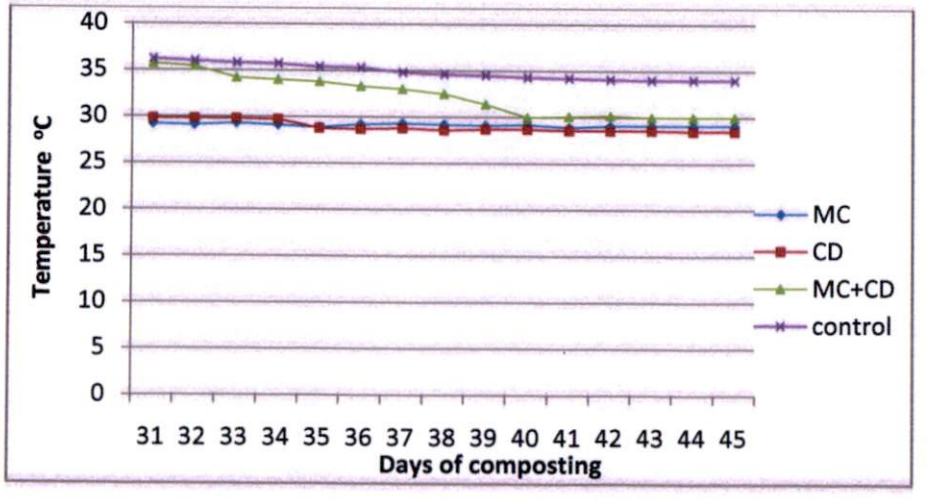




MC –Microbial consortium, CD – Cow dung, MC + CD - Microbial consortium + Cow dung

**Fig 2. Temperature variations during composting of coir pith**

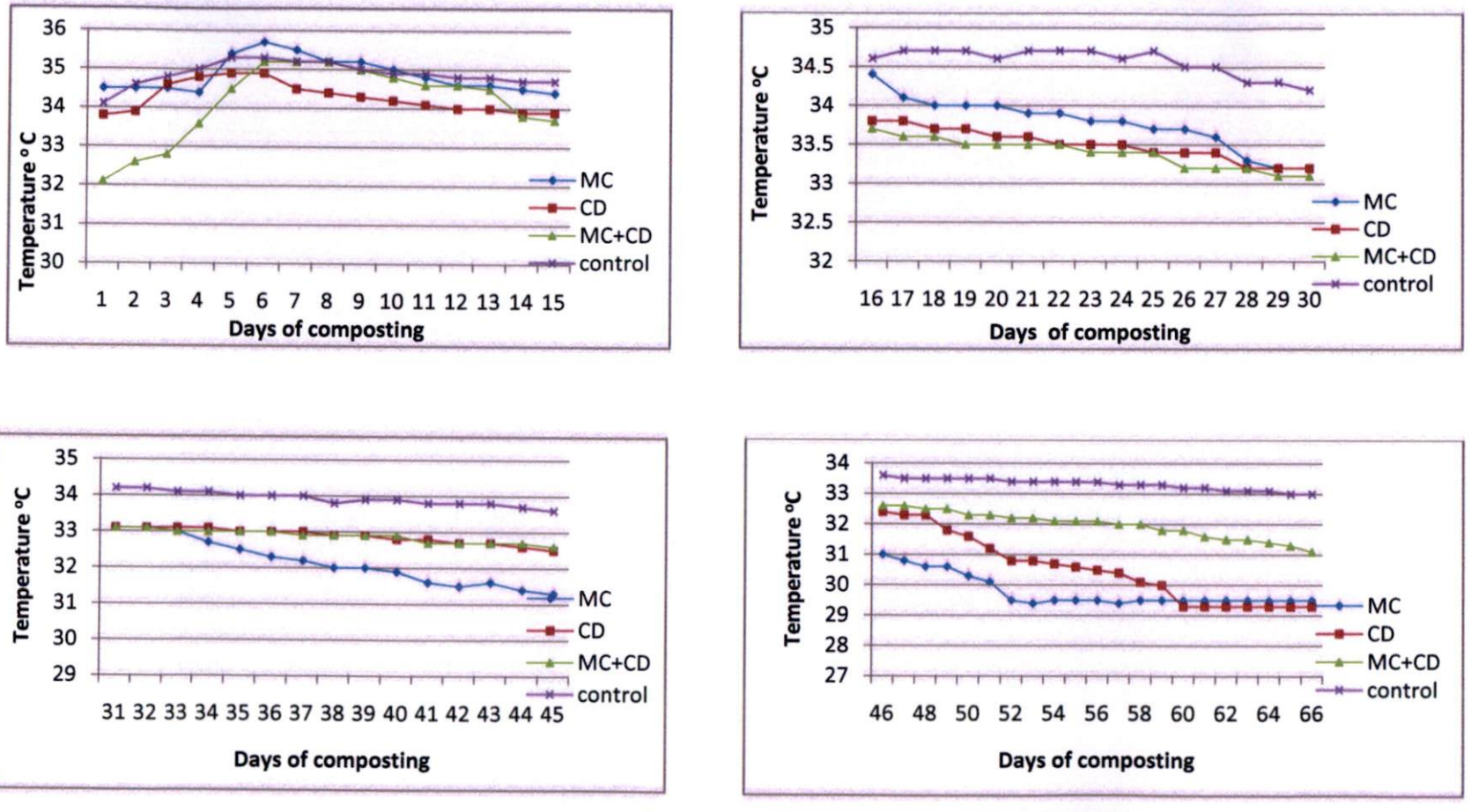


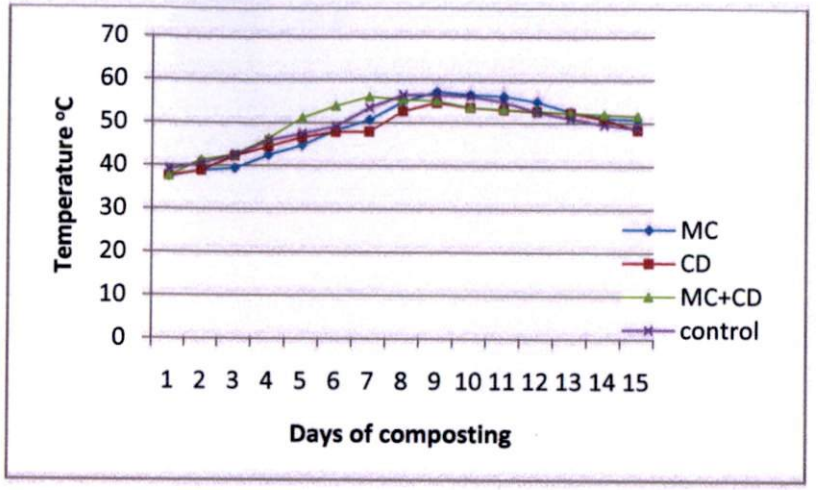

MC –Microbial consortium, CD – Cow dung, MC + CD - Microbial consortium + Cow dung

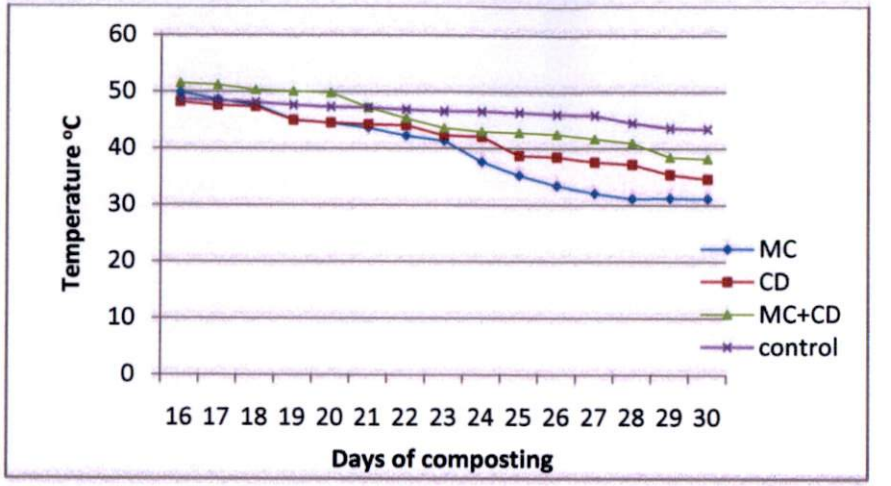
**Fig 2. Temperature variations during composting of coir pith**

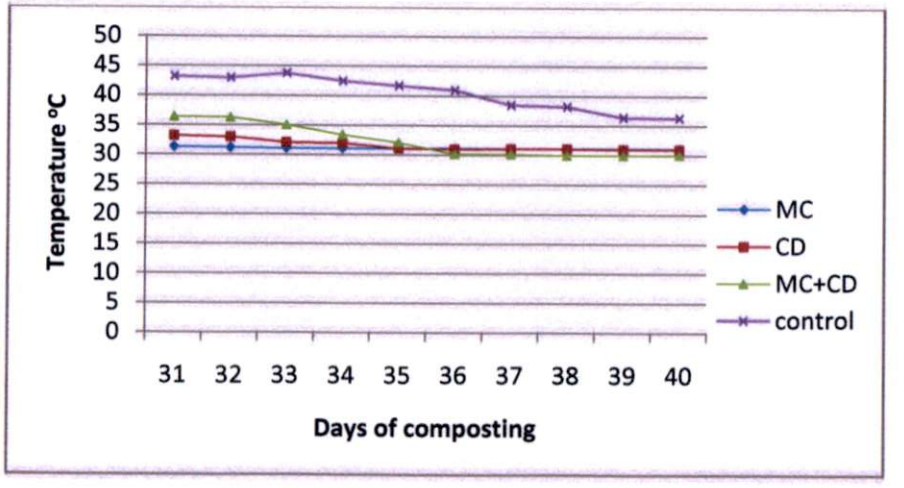



MC –Microbial consortium, CD – Cow dung, MC + CD - Microbial consortium + Cow dung

**Fig 3. Temperature variations during composting of leaf litters**




MC –Microbial consortium, CD – Cow dung, MC + CD - Microbial consortium + Cow dung

**Fig 4. Temperature variations during composting of mixed substrate**

composting with microbial consortium alone. The temperature in different treatments later decreased to 29.3 - 31.1°C at maturity.

As observed in ayurvedic waste, high initial temperature was noticed in mixed substrate which ranged from 37.5 - 39.2°C. The temperature peak of 57.2 °C was noted on 9th day in the treatment with microbial consortium and lowest temperature in various treatments were recorded at maturity which ranged from 30.2 -31.2 °C.

**4.13.2 . Variation in pH during composting**

Variation in the pH observed during the composting period is presented in Table 27. It is observed that, initially all substrates showed acidic pH except coir pith and those added with cow dung slurry which recorded neutral pH. In general, irrespective of the treatments and substrates, pH was found to be increasing during the first and second fortnight, then decreased and later stabilised in alkaline pH at final stages of composting and ranged from 7.6 – 8.6 in various composts. The highest pH (8.6) was observed in ayurvedic compost and leaf litters treated with microbial consortium alone.

## 4.14.*IN VITRO* STUDY ON COMPATIBILITY OF MICROBIAL DEGRADERS WITH COW DUNG

Large scale composting of ligno-phenolic substrates with combination treatment of cow dung slurry and microbial consortium showed an increase in the composting period as compared to the treatment with cow dung slurry alone. Hence, an *in vitro* compatibility study was conducted with selected degraders and 20 per cent cow dung slurry and supernatant in PDA medium (Plate 8 A) and the results revealed that, the native microbes of cow dung were inhibited by certain degraders and *vice versa*. The degraders, *P. variotii*, *S. niveus*, and *Massilia* sp., inhibited the microbes in cow dung whereas, only *Penicillium* spp. were inhibited by cow dung microbes. However, no inhibiton or lysis was observed with other degraders.

## Table 27. Changes in pH during composting of various substrates

| Sl. No. | Composts | Treatments | 0 DAI | 15 DAI | 30 DAI | 45 DAI | 60 DAI |
|---|---|---|---|---|---|---|---|
| 1 | Ayurvedic compost | Consortium alone | 5.3 | 6.2 | 8.3 | 8.1 | 8.6 |
| | | Consortium + Cow dung | 6.6 | 6.8 | 8.5 | 8.6 | 8.0 |
| | | Cow dung alone | 6.0 | 6.5 | 7.9 | 7.8 | 7.7 |
| | | Control | 5.2 | 7.4 | 8.4 | 8.2 | 7.8 |
| 2 | Coir pith compost | Consortium alone | 7.2 | 7.5 | 8.0 | 7.6 | 7.6 |
| | | Consortium + Cow dung | 7.5 | 7.4 | 8.0 | 7.9 | 7.8 |
| | | Cow dung alone | 7.7 | 7.5 | 8.2 | 8.0 | 7.8 |
| | | Control | 7.4 | 7.5 | 8.0 | 7.9 | 7.9 |
| 3 | Mixture compost | Consortium alone | 6.7 | 7.1 | 8.3 | 8.0 | 8.0 |
| | | Consortium + Cow dung | 7.4 | 7.5 | 8.5 | 8.0 | 7.8 |
| | | Cow dung alone | 7.3 | 7.6 | 8.1 | 8.0 | 7.9 |
| | | Control | 6.5 | 7.3 | 7.8 | 8.3 | 8.1 |
| 4 | Leaf litter compost | Consortium alone | 6.8 | 7.2 | 7.9 | 8.4 | 8.6 |
| | | Consortium + Cow dung | 7.1 | 7.2 | 8.1 | 8.4 | 8.5 |
| | | Cow dung alone | 7.2 | 7.8 | 8.4 | 8.2 | 8.2 |
| | | Control | 6.7 | 6.7 | 7.8 | 8.1 | 8.5 |
| 5 | Ordinary compost | Cow dung alone | 7.6 | 7.8 | 8.1 | 7.4 | 7.4 |

DAI – Days after inoculation

*Mean of three replications

## 4.15. REISOLATION OF DEGRADERS FROM MATURE COMPOSTS

The inoculated degraders were reisolated from various mature composts on their respective selective medium confirming their survivability throughout the composting period. On reisolation, all substrates yielded all types of degraders on their respective media and population of cellulose degraders were higher as compared to lignin - tannin degraders. Among the cellulose degraders, bacteria and actinomycetes showed highest count.

## 4.16. CHANGES IN C:N RATIO OF VARIOUS COMPOST PRODUCTS

From the results furnished in Table 28, it is noted that the initial C:N ratio of various substrates ranged from 39.3 - 165.7:1 which showed reduction at maturity, irrespective of treatments. Among the three treatments, application of microbial consortium alone showed lowest C:N ratio recording 47 - 81 per cent reduction from the initial. Among the various composts, ayurvedic compost showed lowest C:N ratio (10.4:1) followed by mixture compost (17.5:1).

Same trend was observed with the combination treatment of cow dung slurry with microbial consortium and cow dung slurry alone. Of these two, cow dung slurry and microbial consortium combination showed lowest C:N ratio as compared to cow dung slurry alone.

## 4.17. NUTRIENT CONTENTS OF VARIOUS COMPOSTS PRODUCTS

Analysis of macro and micronutrient contents of various substrates were carried out before and after composting adopting standard protocols outlined in methodology.

## Table 28. C:N ratio of various compost products

| Substrate | Initial C:N ratio | *C:N ratio at maturity period | | | |
|---|---|---|---|---|---|
| | | Microbial consortium alone (20%) | Microbial consortium + cow dung (20%) | Cow dung (20 %) | Control |
| Ayurvedic waste | 57.7 | 10.4 | 13.9 | 19.3 | 24.1 |
| Coir pith | 165.7 | 33.2 | 41.4 | 53.7 | 125.1 |
| Leaf litter | 39.3 | 20.8 | 30.9 | 33.4 | 35.2 |
| Mixed substrate | 53.5 | 17.5 | 21.9 | 20.2 | 23.7 |
| Ordinary crop residues (check) | 20.8 | - | - | 11.4 | - |

*Mean of three replications

**a. Macronutrient contents of various composts**

Various substrates and mature composts were analysed for nitrogen (N), phosphorus (P) and potassium (K) contents and the results are presented in Table 29, which showed that N, P and K contents of various substrates varied from 2.52 - 2.65, 0.08 - 0.36 and 0.53 -1.24 per cent respectively. At maturity, the NPK contents of all substrates recorded an increase, irrespective of the treatments.

Among the treatments, maximum increase was noted in the substrates treated with microbial consortium alone and ranged from 0.98- 4.80, 0.21- 0.46 and 1.26 -1.60 per cent respectively with maximum contents of nitrogen (4.80 %) and phosphorus (0.46 %) recorded in ayurvedic compost followed by mixture compost with 2.77 per cent nitrogen and leaf litter with 0.42 per cent phosphorus. Whereas, potassium content was found maximum in coir pith compost (1.60 %) followed by ayurvedic compost (1.56 %).

Same trend was observed in the NPK contents of substrates treated with cow dung slurry alone and in combination with consortium, in which the highest contents of N and P were noted in ayurvedic compost. However, coir pith recorded maximum K content with combination of consortium and cow dung slurry, whereas, ayurvedic compost showed maximum K content on treatment with cow dung slurry alone.

**b. Secondary and micronutrient contents of various composts**

Micronutrients *viz.* calcium, magnesium, copper, manganese, zinc and iron in the substrates composted with microbial consortium was analysed at maturity and presented in Table 30.

Micronutrients such as copper, manganese, zinc and iron were present in low concentration in various composts. Among the composts, noticeable difference was observed only with copper and manganese contents. Maximum

## Table 29. Macronutrient contents of various composts

| Composts | * Nitrogen (%) | | | | | * Phosphorus (%) | | | | | * Potassium (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | Microbial consortium alone | Microbial consortium + cow dung | Cow dung alone | Control | Initial | Microbial consortium alone | Microbial consortium + cow dung | Cow dung alone | Control | Initial | Microbial consortium alone | Microbial consortium + cow dung | Cow dung alone | Control |
| Ayurvedic compost | 2.52 | 4.8 | 3.57 | 3.62 | 2.7 | 0.36 | 0.46 | 0.41 | 0.41 | 0.38 | 1.03 | 1.56 | 1.33 | 1.30 | 1.29 |
| Coir pith compost | 0.34 | 0.98 | 0.59 | 1.07 | 0.45 | 0.08 | 0.21 | 0.20 | 0.19 | 0.19 | 0.53 | 1.60 | 1.5 | 1.16 | 0.74 |
| Leaf litter compost | 1.09 | 1.80 | 1.72 | 1.52 | 1.30 | 0.17 | 0.35 | 0.33 | 0.30 | 0.24 | 0.90 | 1.26 | 1.26 | 1.20 | 0.97 |
| Mixture compost | 2.05 | 2.77 | 2.28 | 2.20 | 2.18 | 0.30 | 0.34 | 0.34 | 0.32 | 0.31 | 1.24 | 1.60 | 1.44 | 1.39 | 1.30 |
| Ordinary compost | 2.65 | - | - | 2.8 | - | 0.35 | - | - | 0.37 | - | 1.01 | - | - | 1.54 | - |

* Mean of three replications

**Table 30. Secondary and micronutrient contents of various composts**

| Composts | * Calcium (%) | Magnesium (%) | Copper (ppm) | Manganese (ppm) | Zinc (ppm) | Iron (ppm) |
|---|---|---|---|---|---|---|
| Ayuvedic compost | 0.19 | 0.041 | 13.58 | 86.22 | 19.60 | 292.40 |
| Coir pith compost | 0.16 | 0.038 | 5.46 | 91.52 | 14.32 | 234.62 |
| Leaf litter compost | 0.20 | 0.040 | 6.94 | 119.7 | 11.24 | 235.04 |
| Mixture compost | 0.18 | 0.038 | 3.97 | 31.48 | 11.22 | 223.12 |
| Ordinary compost | 0.20 | 0.040 | 8.34 | 54.06 | 16.84 | 263.76 |

* Mean of three replications

contents of magnesium (0.04 %), copper (13.58 ppm), zinc (19.60 ppm) and iron (292.40 ppm) were observed in ayurvedic compost whereas, calcium (0.2 %) and manganese (119.7 ppm) were found to be highest in leaf litter compost.

## 4.18. HEAVY METAL CONTENTS IN VARIOUS COMPOSTS

Analysis of heavy metals showed absence of cadmium, arsenic and mercury in all compost samples. However, traces of chromium (4.0 ppm) and nickel (2.7 ppm) were detected in ayurvedic compost and lead in all the samples (0.84 - 30.88 ppm) which were lower than maximum permissible limits of 50 mg/kg for both chromium and nickel and 100 mg/kg for lead (Table 31).

## 4.19. BIOCHEMICAL PROPERTIES OF VARIOUS COMPOSTS

Data on the analysis of biochemical components of various substrates before and after composting with microbial consortium are furnished in Table 32. It is evident that, various substrates showed drastic reduction in cellulose, lignin and tannin contents on composting with microbial consortium.

Among the substrates, ayurvedic waste recorded highest initial cellulose content (35.70 %) which reduced to 18.07 per cent with maximum reduction of 49.38 per cent. Lignin and tannin contents also recorded 47.68 and 95.21 per cent reduction respectively on composting with microbial consortium.

In case of coir pith, the highest lignin content of 51.52 per cent was recorded initially, which reduced to 26.26 per cent with a reduction of 49.02 per cent on composting. Cellulose and tannin contents showed reduction of 37.44 and 93.19 per cent respectively.

Leaf litter recorded maximum reduction in tannin content among the substrates, which reduced from 28.46 to 0.40 per cent with 98.59 per cent

**Table 31. Heavy metal contents in various composts**

| Composts | * Lead(Pb) | * Cadmium (Cd) | * Arsenic (As) | * Mercury (Hg) | * Cromium (Cr) (ppm) | * Nickel (Ni) (ppm) |
|---|---|---|---|---|---|---|
| Permissible limits | 100mg/kg | 5mg/kg | 10mg/kg | 0.15 mg/kg | 50mg/kg | 50mg/kg |
| Ayurvedic compost | 30.88 | 0 | 0 | 0 | 4.00 | 2.74 |
| Coir pith compost | 11.54 | 0 | 0 | 0 | 0 | 0 |
| Leaf litter compost | 0.84 | 0 | 0 | 0 | 0 | 0 |
| Mixture compost | 13.54 | 0 | 0 | 0 | 0 | 0 |
| Ordinary compost | 13.5 | 0 | 0 | 0 | 0 | 0 |

* Mean of three replications

## Table 32. Biochemical analysis of various composts

| Composts | * Cellulose (%) | | | * Lignin (%) | | | * Tannin (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Per cent reduction | Initial | Final | Per cent reduction | Initial | Final | Per cent reduction |
| Ayurvedic compost | 35.70 | 18.07 | 49.38 | 37.6 | 19.67 | 47.68 | 24.23 | 1.16 | 95.21 |
| Coir pith compost | 32.00 | 20.02 | 37.44 | 51.52 | 26.26 | 49.02 | 15.14 | 1.03 | 93.19 |
| Leaf litter compost | 33.36 | 20.14 | 39.62 | 24.46 | 23.75 | 2.91 | 28.46 | 0.40 | 98.59 |
| Mixture compost | 24.07 | 17.67 | 26.58 | 22.55 | 19.86 | 11.92 | 15.16 | 0.72 | 95.25 |
| Ordinary compost | 33.43 | 25.04 | 25.09 | NA | NA | NA | NA | NA | NA |

NA – Not analysed

* Mean of three replications

reduction. Whereas, cellulose and lignin contents of leaf litter recorded 39.62 and 2.91 per cent reduction respectively.

On composting with microbial consortium, mixed substrate showed 26.58, 11.92 and 95.25 per cent reduction of cellulose, lignin and tannin respectively.

## 4.20. LARGE SCALE COMPOSTING OF VARIOUS SUBSTRATES WITH TALC BASED CONSORTIAL FORMULATION

Composting of various substrates using talc based formulation of microbial consortium were conducted during April - May and October – November and data are shown in Table 33. The results presented in the table showed variation in the maturity periods, recording 40, 40, 45, 60 days (April - May) and 32, 34, 34, 57 days (October - November) in mixed substrate, coir pith, ayurvedic waste and leaf litters respectively, which showed slight early maturity during rainy period as compared to summer.

At maturity, weight reduction of 50 – 72 per cent was recorded in April – May and 46 - 54 per cent in October – November months. In both seasons, ayurvedic waste showed maximum weight reduction.

Summing up the findings on the effect of microbial consortium on various substrates, under *in vitro, in vivo* and large scale composting with different treatments including microbial consortium alone, cow dung slurry alone and the combination of both, showed that, microbial consortium alone (liquid or talc based formulation) was the most effective one in reducing the composting period. Of the different substrates tested, early maturity was observed in mixed substrates and longest with leaf litters of cashew, teak and mango irrespective of treatments.

**Table 33. Large scale composting of various substrates with talc formulation of microbial consortium**

| Sl. No. | Substrates | * Maturity period (days) | | * Weight of composts (kg) | | | |
|---|---|---|---|---|---|---|---|
| | | | | April - May | | October -November | |
| | | Consortium alone (20%) (April - May) | Consortium alone (20 %) (Oct. – Nov.) | Initial | Final | Initial | Final |
| 1 | Ayurvedic waste | 45 | 34 | 50 | 14 | 50 | 23 |
| 2 | Coir pith | 40 | 34 | 50 | 16 | 50 | 27 |
| 3 | Leaf litter | 60 | 57 | 15 | 7.5 | 15 | 8 |
| 4 | Mixed substrates | 40 | 32 | 50 | 15.5 | 50 | 25 |

*Mean of three replications

## 4.21. SCREENING FOR THERMOTOLERANCE POTENTIAL MICROBIAL DEGRADERS

Sampling of the cultures of degraders incubated at 40 - 60°C showed viable colonies on PDA mediated plates at 2 and 4 days after incubation. However, no growth was observed in the samples kept at 65 °C (Table 34). This study indicated that, all degraders selected for microbial consortium were able to withstand to 60 °C and are thermophilic in nature.

## 4.22. EXTRA CELLULAR ENZYME ACTIVITY OF SELECTED DEGRADERS

From the results presented in Table 35 it is found that, all the degraders selected for microbial consortium showed activity of different extra cellular enzymes in varying proportions (Plate 8B).

It is observed from the data that, all tested degraders exhibited cellulase activity with maximum by *T. harzianum* followed by *P. oxalicum* and *P. chrysogenum.* All degraders except, *P. variotii, S. albus* and *Candida* sp. recorded amylase activity and maximum by *Arthrobacter* sp. followed by *S. niveus.* None of the degraders except *P. variotii* showed activity of xylanase and protease activity was observed only in *P. oxalicum*, *S. niveus* and *Arthrobacter* sp. In case of catalase, only bacteria, actinomycetes and yeast showed positive reaction. All fungal degraders showed lignin peroxidase activity in addition to *S. niveus* and *Massilia* sp. Polyphenol oxidase activity was recorded by all degraders except *T. harzianum, S. niveus*, *Arthrobacter* sp. and *Massilia* sp.

From the above results, it is revealed that, among the tested degraders, *P. oxalicum* and *S. niveus* showed maximum number of enzyme activity.

**Table 34. Screening for thermotolerance potential of selected microbial degraders**

| Sl No. | Isolates | * Thermotolerance at (°C) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 40 | 45 | 50 | 55 | 60 | 65 |
| 1 | *Trichoderma harzianum* | + | + | + | + | + | - |
| 2 | *Paceilomyces variotii* | + | + | + | + | + | – |
| 3 | *Penicillium oxalicum* | + | + | + | + | + | – |
| 4 | *Talaromyces* sp. | + | + | + | + | + | – |
| 5 | *Penicillium chrysogenum* | + | + | + | + | + | – |
| 6 | *Streptomyces niveus* | + | + | + | + | + | – |
| 7 | *Streptomyces albus* | + | + | + | + | + | – |
| 8 | *Streptomyces albus* | + | + | + | + | + | – |
| 9 | *Arthrobacter* sp. | + | + | + | + | + | – |
| 10 | *Massilia* sp. | + | + | + | + | + | – |

+ Positive -- Negative

* Mean of three replications

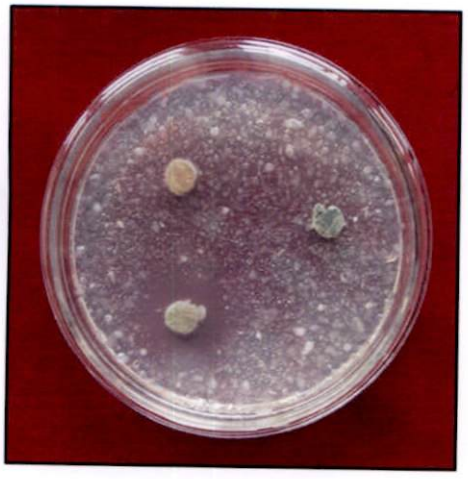
## Table 35. Extra cellular enzymatic activity of selected degraders

| Sl. No. | Isolate | * Cellulase | Amylase | Xylanase | Protease | Catalase | Lignin peroxidase | Polyphenol oxidase |
|---|---|---|---|---|---|---|---|---|
| 1 | *Trichoderma harzianum* | +++ | + | – | – | – | + | - |
| 2 | *Paceilomyces variotii* | + | – | + | – | – | + | ++ |
| 3 | *Penicillium oxalicum* | ++ | + | – | + | – | + | + |
| 4 | *Talaromyces* sp. | + | + | – | – | – | + | + |
| 5 | *Penicillium chrysogenum* | ++ | + | – | – | - | + | + |
| 6 | *Streptomyces niveus* | + | ++ | - | + | + | + | – |
| 7 | *Streptomyces albus* | + | – | – | – | + | – | + |
| 8 | *Streptomyces albus* | + | – | – | – | + | – | + |
| 9 | *Arthrobacter* sp. | + | +++ | - | + | + | - | - |
| 10 | *Massiila* sp. | + | ++ | – | – | + | + | – |
| 11 | *Candida* sp. | + | – | – | – | + | – | + |

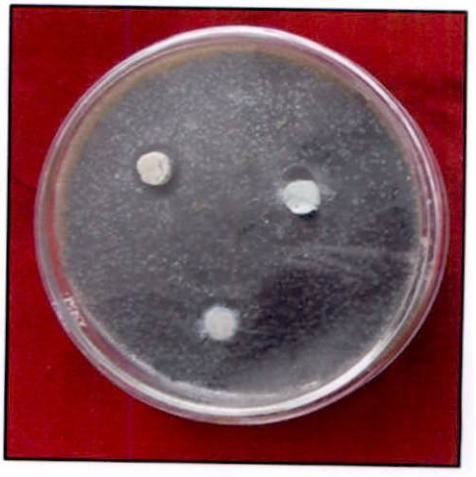
High +++, Medium ++ , Poor +, Nil – , * Mean of three replications

# PLATE 8

## A. COMPATIBILITY OF MICROBIAL DEGRADERS WITH COW DUNG

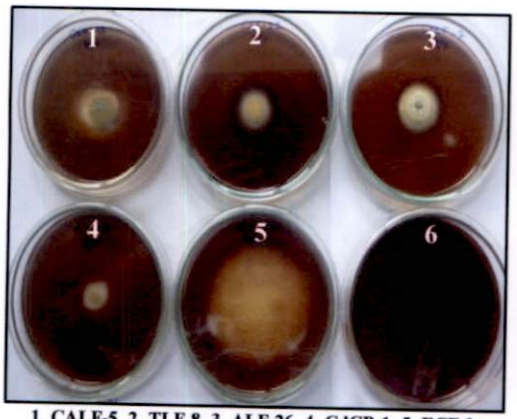


With cow dung supernatant



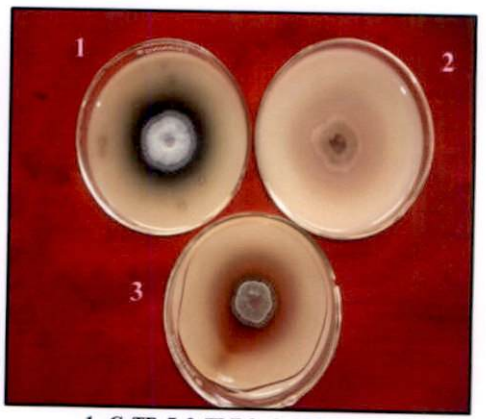
With cow dung slurry

## B. EXTRA CELLULAR ENZYME ACTIVITY OF SELECTED DEGRADERS



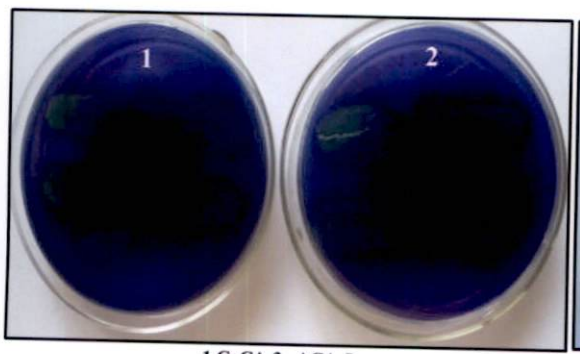

1. CALF-5, 2. TLF-8, 3. ALF-26, 4. CdCB-1, 5. ECF-2, 6. Control

Cellulase




1. CaTF- 7, 2. TLF-8, 3. ALF-26

Polyphenol oxidase




1.CaCA-3, ACA-5

Lignin peroxidase


1.ACA-5, 2.TLF-8, 3. CCB-8, 4. CdCB-1, 5. CaTF-7, 6.Control

Amylase

## 4. 23. *IN VITRO* SCREENING OF SELECTED MICROBIAL DEGRADERS AGAINST SOIL BORNE PATHOGENS

Degraders selected for microbial consortium were tested for their antagonistic property against six major soil borne pathogens and the data are given in Table 36.

It is observed from the data that, all degraders selected for consortium have antagonistic property against one or another soil borne pathogens. However, per cent inhibition varied with the degraders. All degraders showed antagonistic activity on *Rhizoctonia* sp. and *Fusarium* sp., but the inhibitory effect was less. Maximum inhibition was recorded by *T. harzianum* on *Rhizoctonia* sp. with 47.27 per cent and 44.62 per cent by *P. oxalicum* on *Fusarium* sp.

All degraders except, *Arthrobacter* sp. and *Candida* sp. showed antagonistic activity on *Phytophthora* sp. recording 62.78 - 22.22 per cent inhibition with maximum by *T. harzianum.* In case of *Pythium* sp., none of the bacteria and actinomycetes showed antagonistic property and only the fungal degraders were found to be effective against this pathogen recording 60.00 - 44.44 per cent inhibition, with maximum by *T. harzianum.*

With regard to *Sclerotium* sp., only *T. harzianum, P. variotii* and *P. oxalicum* showed inhibition. Whereas, in case of *Ralstonia solanacaearum*, except bacterial antagonists, all others showed antagonistic property, with maximum by *T .harzianum* (50 %) and *P. variotii* (46.66 %).

Considering the overall performance of the selected degraders against the soil borne pathogens, fungal degraders were more effective than bacteria and actinomycetes. Among the fungal degraders, *T. harzianum* was found to be the most effective antagonist. It is also noted that, the bacterium, *Massilia* sp. showed good inhibitory effect on *Rhizoctonia* sp. *and Phytophthora* sp. (Plate 9).

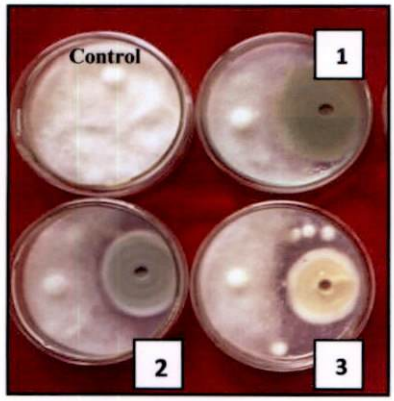
**Table 36. *In vitro* screening of microbial degraders against soil borne pathogens**

| Sl. No. | Microbial degraders | *Per cent Inhibition | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Rhizoctonia* sp. | *Fusarium* sp. | *Pythium* sp. | *Phytophthora* sp. | *Sclerotium* sp. | *Ralstonia solanacearum* |
| 1 | *Trichoderma harzianum* | 47.27 | 35.71 | 60.00 | 62.78 | 41.66 | 50.00 |
| 2 | *Paceilomyces variotii* | 29.99 | 33.03 | 58.30 | 52.75 | 16.66 | 46.66 |
| 3 | *Penicillium oxalicum* | 36.10 | 44.62 | 44.44 | 50.55 | 11.11 | 12.22 |
| 4 | *Talaromyces* sp. | 27.27 | 26.08 | 50.00 | 54.44 | 0 | 14.44 |
| 5 | *Penicillium chrysogenum* | 21.81 | 15.21 | 55.50 | 49.44 | 0 | 11.11 |
| 6 | *Aspergillus nidulans* | 25.45 | 33.03 | 58.30 | 49.44 | 0 | 30.55 |
| 7 | *A. sclerotiorum* | 25.45 | 38.39 | 54.95 | 54.99 | 0 | 43.88 |
| 8 | *Streptomyces niveus* | 10.90 | 15.17 | 0 | 44.44 | 0 | 14.44 |
| 9 | *Streptomyces albus* | 22.73 | 15.17 | 0 | 22.22 | 0 | 15.55 |
| 10 | *Arthrobacter* sp. | 19.99 | 13.39 | 0 | 0 | 0 | 0 |
| 11 | *Massilia* sp. | 43.63 | 6.24 | 0 | 39.44 | 0 | 0 |
| 12 | *Candida* sp. | 22.72 | 2.33 | 0 | 0 | 0 | 16.66 |

* Mean of three replications

# PLATE 9

## *IN VITRO* SCREENING OF SELECTED DEGRADERS AGAINST SOIL BORNE PATHOGENS

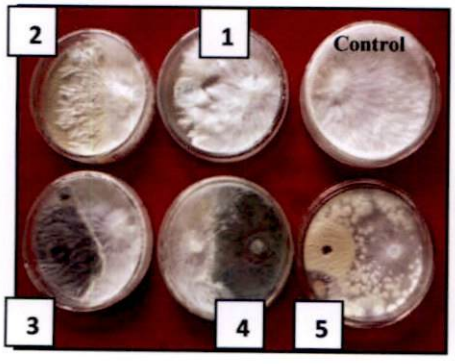



**1**. CaLF-5 **2.**CaTF -7 **3.**TLF-8
**X**
***Pythium* sp.**


**1** .CaTF-7 **2.**ECF-2 **3.**CaLF-5 **4.**CaTF -7 **5.**TLF-8
**X**
***Phytophthora* sp.**

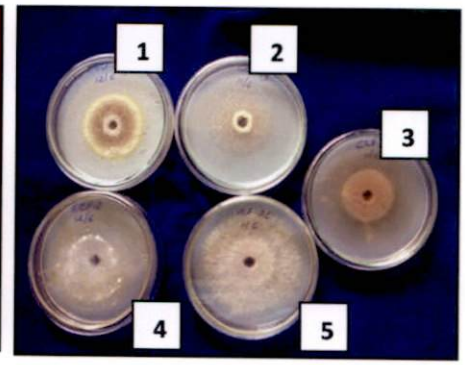

**1**. CaLF-7 **2.**ECF -2 **3.**CaLF-5
**X**
***Rhizoctonia* sp.**


**1.** CaLF-7 **2.** ECF-2 **3**.TLF-8 **4.** ALF-26
**X**
***Fusarium* sp.**




**1** .ALF-26 **2.**CaLF-5 **3.**CaLF-7 **4.**ECF-2 **5.**TLF-8

X
***Sclerotium* sp.**




**1.** ALF-26 **2.** CaTF-7 **3**. TLF-8 **4**.CaLF-7 **5**. ECF- 2

X
***Ralstonia solanacearum***

## 4.24. BIOEFFICACY OF LIGNO-PHENOLIC COMPOST PRODUCTS ON THE MANAGEMENT OF LEAF BLIGHT OF AMARANTH

A field experiment was conducted to evaluate the efficacy of various compost products on the management of leaf blight of amaranth caused by *Rhizoctonia solani* (Plate 10). Observations on disease incidence/severity (30 and 60 DAP), plant height and yield / plot were recorded. The results of the experiment are summarised in Tables 37 and 38.

From the data presented in Table 37, it is noticed that, disease incidence was comparatively higher in all treatments and the lowest incidence (66.67 and 73.40 %) was observed in the treatment ($T_8$), mixture compost applied in two splits at 30 and 60 DAP.

At 30 DAP disease severity was also comparatively higher in all treatments recording 22.29 – 57.26 per cent against 70.51 per cent in control. Minimum severity (22.29 %) was observed in ayurvedic compost applied in splits ($T_2$) which was on par with coir pith compost applied as full basal -$T_3$ (22.66 %) and mixture compost applied as splits -$T_8$ (23.85 %) which showed 68.39, 67.86. and 66.18 per cent reduction over control respectively.

The infection was found to be reduced on new flushes after harvest and significant difference was noticed among the treatments at 60 DAP. The minimum severity (13.61 %) was recorded in $T_2$ which was on par with $T_1$, ayurvedic compost applied as full basal (14.81 %) and $T_8$, mixture compost applied in splits (15.89 %) with 65.88, 62.87 and 60.17 per cent reduction over control respectively. It is also noted that, application of compost products showed less severity as compared to chemical fertilizer and cow dung treated ones and the split application was more effective than full basal application.

**Table 37. Efficacy of various composts on the management of leaf blight of amaranth**

| Tr . No. | Treatment details | 30 DAP | 60 DAP | 30 DAP | Per cent reduction over control | 60 DAP | Per cent reduction over control |
|---|---|---|---|---|---|---|---|
| $T_1$ | Ayurvedic compost ( Full basal) | $100^{a}$ (10.03) | $98.55^{a}$ (9.95) | $30.66^{efg}$ (5.57) | 56.51 | $14.81^{fg}$ (3.9) | 62.87 |
| $T_2$ | Ayurvedic compost (Two splits ) | $90.47^{ab}$ (9.51) | $85.92^{a}$ (9.28) | $22.29^{g}$ (4.74) | 68.39 | $13.61^{g}$ (3.72) | 65.88 |
| $T_3$ | Coir pith compost ( Full basal) | $91.67^{ab}$ (9.60) | $90.90^{a}$ (9.52) | $22.66^{g}$ (4.79) | 67.86 | $19.67^{defg}$ (4.49) | 50.69 |
| $T_4$ | Coir pith compost (Two splits ) | $91.41^{ab}$ (9.58) | $94.44^{a}$ (9.74) | $34.66^{def}$ (5.89) | 50.84 | $17.29^{defg}$ (4.22) | 56.66 |
| $T_5$ | Leaf litter compost ( Full basal) | $100^{a}$ (10.03) | $100^{a}$ (10.03) | $43.85^{bcde}$ (6.66) | 37.81 | $19.99^{defg}$ (4.51) | 49.89 |
| $T_6$ | Leaf litter compost (Two splits ) | $98.33^{a}$ (9.94) | $98.48^{a}$ (9.95) | $41.85^{bcde}$ (6.49) | 40.65 | $17.55^{defg}$ (4.23) | 56.00 |
| $T_7$ | Mixture compost ( Full basal) | $100^{a}$ (10.03) | $96.97^{a}$ (9.87) | $35.88^{cdef}$ (6.02) | 49.11 | $22.67^{cde}$ (4.79) | 43.17 |
| $T_8$ | Mixture compost (Two splits ) | $66.67^{c}$ (8.08) | $73.40^{b}$ (8.55) | $23.85^{fg}$ (4.93) | 66.18 | $15.89^{efg}$ (4.02) | 60.17 |
| $T_9$ | Aerobic compost ( Full basal) | $76.91^{bc}$ (8.77) | $94.67^{a}$ (9.75) | $44.66^{bcd}$ (6.72) | 36.66 | $27.99^{bc}$ (5.34) | 29.83 |
| $T_{10}$ | Aerobic compost (Two splits ) | $100^{a}$ (10.03) | $100^{a}$ (10.03) | $55.21^{ab}$ (7.39) | 21.70 | $24.32^{cd}$ (4.97) | 39.03 |
| $T_{11}$ | Cow dung @50t/ha ( Full basal) | $100^{a}$ (10.03) | $100^{a}$ (10.03) | $57.26^{ab}$ (7.59) | 18.79 | $36.99^{a}$ (6.12) | 7.27 |
| $T_{12}$ | Cow dung @50t/ha(Two splits ) | $100^{a}$ (10.03) | $100^{a}$ (10.03) | $49.92^{bc}$ (7.09) | 29.20 | $32.66^{ab}$ (5.74) | 18.12 |
| $T_{13}$ | *Pseudomonas fluorescens* @2.0 % | $100^{a}$ (10.03) | $93.34^{a}$ (9.68) | $35.22^{def}$ (5.97) | 50.05 | $21.22^{cdef}$ (4.66) | 46.80 |
| $T_{14}$ | NPK as per POP | $100^{a}$ (10.03) | $100^{a}$ (10.03) | $56.10^{ab}$ (7.51) | 20.44 | $37.66^{a}$ (6.17) | 5.59 |
| $T_{15}$ | Absolute control | $100^{a}$ (10.03) | $100^{a}$ (10.03) | $70.51^{a}$ (8.42) | - | $39.89^{a}$ (6.36) | - |
| CD (0.05) | | **0.95** | **0.71** | **0.99** | - | **0.73** | - |

* Mean of three replications DAP- Days after planting Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values

Figures followed by same letter do not differ significantly according to DMRT

# PLATE 10

## EFFICACY OF COMPOSTS ON THE MANAGEMENT OF LEAF BLIGHT OF AMARANTH


**General view of the field**

**Treated plot**


**Treated plot**

**Leaf blight infection**

With regard to plant height and yield (Table 38), $T_{11}$- cow dung applied as basal, recorded maximum plant height (39.95 cm) and yield (5.10kg/5M$^2$) which was on par with $T_2$ - ayurvedic compost applied in splits, recording plant height of 38.45 cm and a yield of 4.33kg/5m$^2$. In addition, $T_{13}$ - *Pseudomonas fluorescens* (2 %) was also equally effective with 38.20 cm, plant height and 3.90 kg/5m$^2$ yield.

## 4.25. BIOEFFICACY OF LIGNO-PHENOLIC COMPOST PRODUCTS ON THE MANAGEMENT OF BACTERIAL WILT OF TOMATO

Bioefficacy of various lignin- tannin rich compost products were evaluated against bacterial wilt of tomato caused by *Ralstonia solanacearum* (Plate 11). Observations on wilt incidence and biometric characters were recorded at 30, 45 and 60 days after planting. The experimental findings are detailed in Table 39, 40 and 41).

It appeared from Table 39 that, per cent wilt incidence at 30 DAP showed no significant difference among the treatments. However, all treatments were superior to control at 45 and 60 DAP with minimum incidence of 4.16 per cent and 6.93 per cent in check plot - $T_{13}$ (soil drenching with copper hydroxide, 2g/l). Among the other treatments, lowest incidence was observed in split application of ayurvedic compost - $T_2$ with 6.94 per cent against 36.10 per cent in control, recording 80.77 per cent disease reduction.

At 60 DAP, same trend was noticed with minimum disease incidence in $T_2$, split application of ayurvedic compost (13.88 %) which was on par with full basal application of leaf litter compost - $T_5$ (15.27 %), ayurvedic compost -$T_1$ (16.66 %) and coir pith compost -$T_3$ (16.66 %) against 41.68 per cent in control and these treatments showed 86.79 to 60.02 per cent reduction of wilt incidence.

**Table 38. Effect of various composts on plant height and yield of amaranth**

| Treatments | Treatment details | * Plant height (cm) (30 DAP) | * Yield/5M$^2$ (kg) |
|---|---|---|---|
| $T_1$ | Ayurvedic compost ( Full basal) | $32.25^{bc}$ | $2.93^{bcd}$ |
| $T_2$ | Ayurvedic compost (Two splits ) | $38.45^{a}$ | $4.33^{ab}$ |
| $T_3$ | Coir pith compost ( Full basal) | $23.20^{fg}$ | $2.00^{d}$ |
| $T_4$ | Coir pith compost (Two splits ) | $24.5^{efg}$ | $2.83^{cd}$ |
| $T_5$ | Leaf litter compost ( Full basal) | $28.45^{bcde}$ | $2.00^{d}$ |
| $T_6$ | Leaf litter compost (Two splits ) | $30.90^{bcd}$ | $2.50^{bcd}$ |
| $T_7$ | Mixture compost ( Full basal) | $32.90^{b}$ | $2.50^{bcd}$ |
| $T_8$ | Mixture compost (Two splits ) | $28.55^{bcde}$ | $2.67^{bcd}$ |
| $T_9$ | Aerobic compost ( Full basal) | $26.05^{def}$ | $2.63^{bcd}$ |
| $T_{10}$ | Aerobic compost (Two splits ) | $27.15^{cdef}$ | $3.00^{bcd}$ |
| $T_{11}$ | Cow dung @50t/ha ( Full basal) | $39.95^{a}$ | $5.10^{a}$ |
| $T_{12}$ | Cow dung @50t/ha (Two splits ) | $32.20^{bc}$ | $2.66^{bcd}$ |
| $T_{13}$ | *Pseudomoans fluorescens* @2.0 % | $38.25^{a}$ | $3.90^{ab}$ |
| $T_{14}$ | NPK as per POP | $29.30^{bcde}$ | $3.33^{abc}$ |
| $T_{15}$ | Absolute control | $20.05^{g}$ | $2.00^{d}$ |
| CD (0.05) | | **4.55** | **1.2** |

* Mean of three replications

Figures followed by same letter do not differ significantly according to DMRT

## Table 39. Efficacy of various composts on the management of bacterial wilt of tomato

| Tr . No. | Treatment details | 30 DAP | | 45 DAP | | 60 DAP | |
|---|---|---|---|---|---|---|---|
| | | * Per cent wilt incidence | Per cent reduction over control | * Per cent wilt incidence | Per cent reduction over control | * Per cent wilt incidence | Per cent reduction over control |
| $T_1$ | Ayurvedic compost ( Full basal) | 2.77 | 77.82 | 13.88 $^{bc}$ (3.73) | 61.52 | 16.66$^{cd}$ (3.94) | 60.02 |
| $T_2$ | Ayurvedic compost (Two splits ) | 1.38 | 88.95 | 6.94$^{bc}$ (2.70) | 80.77 | 13.88$^{cd}$ (3.73) | 66.69 |
| $T_3$ | Coir pith compost ( Full basal) | 1.38 | 88.95 | 12.49$^{bc}$ (3.57) | 65.40 | 16.66$^{cd}$ (3.94) | 60.02 |
| $T_4$ | Coir pith compost (Two splits ) | 2.77 | 77.82 | 19.44$^{ab}$ (4.40) | 46.14 | 22.22$^{bc}$ (4.76) | 46.68 |
| $T_5$ | Leaf litter compost ( Full basal) | 5.55 | 55.56 | 13.88$^{bc}$ (3.64) | 22.22 | 15.27$^{cd}$ (3.91) | 63.36 |
| $T_6$ | Leaf litter compost (Two splits ) | 1.38 | 88.95 | 16.66$^{ab}$ (4.07) | 50.00 | 18.05$^{cd}$ (3.94) | 56.69 |
| $T_7$ | Mixture compost ( Full basal) | 1.38 | 88.95 | 19.44$^{ab}$ (4.42) | 46.14 | 23.61$^{bc}$ (4.90) | 43.35 |
| $T_8$ | Mixture compost (Two splits ) | 4.16 | 66.69 | 13.88$^{bc}$ (3.73) | 61.52 | 24.99$^{bc}$ (5.03) | 40.04 |
| $T_9$ | Aerobic compost ( Full basal) | 6.94 | 44.43 | 16.66$^{bc}$ (3.91) | 53.85 | 23.60$^{bc}$ (4.88) | 43.37 |
| $T_{10}$ | Aerobic compost (Two splits ) | 2.77 | 77.82 | 16.66$^{ab}$ (4.12) | 53.85 | 29.16$^{ab}$ (5.42) | 30.01 |
| $T_{11}$ | Cow dung @25t/ha (Full basal) | 2.77 | 77.82 | 20.83$^{ab}$ (4.60) | 42.29 | 22.22$^{bc}$ (4.76) | 46.68 |
| $T_{12}$ | Cow dung @25t/ha (Two splits ) | 2.77 | 77.82 | 18.05$^{ab}$ (4.18) | 50.00 | 30.55$^{ab}$ (5.55) | 26.70 |
| $T_{13}$ | Copper hydroxide -2g/l | 0 | 100 | 4.16$^{c}$ (1.94) | 92.35 | 6.93$^{d}$ (2.97) | 86.70 |
| $T_{14}$ | NPK as per POP | 0 | 100 | 18.05$^{ab}$ (4.26) | 50.00 | 23.61$^{bc}$ (4.90) | 43.35 |
| $T_{15}$ | Absolute control | 12.49 | - | 36.10$^{a}$ (6.06) | - | 41.68$^{a}$ (6.48) | |
| CD (0.05) | | - | | **1.65** | | | **1.25** |

* Mean of three replications DAP - Days after planting Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values

DAP –Days after planting , * Mean of three replications

Figures followed by same letter do not differ significantly according to DMRT

# PLATE 11

## EFFICACY OF COMPOSTS ON THE MANGEMENT OF BACTERIAL WILT OF TOMATO

General view of the field

Treated plot


Treated plots

Wilt infection

In the biometric characters (Table 40), significant difference was noticed only with respect to plant height and among the treatments, application of chemical fertilizers - $T_{14}$ recorded maximum plant height (59.96 cm) followed by the application of NPK with copper hydroxide ($T_{13}$) which were on par with basal application of cow dung and split application of ayurvedic compost ($T_{11}$ and $T_2$). No significant difference was noticed with respect to days to flowering and to first harvest.

With respect to yield parameters, significant difference was noticed with respect to number of fruits per plant, weight of fruits, yield per plant and yield per plot, which varied with the treatments. From the data showed in Table 41, it is found that, among the treatments, NPK application ($T_{14}$) showed maximum number of fruits per plant (20.82) whereas, split application of ayurvedic compost ($T_2$) recorded maximum weight of the fruit (40.36 g). Yield per plant (799.24 g) and yield per plot (12.4 kg/6.48$m^2$) were maximum with NPK application with soil drenching of copper hydroxide ($T_{13}$) followed by 10.9 kg/6.48$m^2$ in NPK application alone ($T_{14}$). Among the different composts, split and basal application of ayurvedic compost ($T_2$ and $T_1$) recorded highest yield of 8.9 kg/ 6.48 $m^2$ which was on par with split application of mixture compost, $T_8$ (8.6kg/6.48 $m^2$).

Summing up the field experiments, it is observed that, lignin- tannin rich compost products were effective in suppressing soil borne diseases. In case of bacterial wilt of tomato, even though the chemical treatment with copper hydroxide showed maximum reduction in wilt incidence, among the organic treatments, application of ayurvedic compost was the most effective one. In case of amaranth leaf blight, all compost treatments were better than cow dung and chemical fertilizer. However, ayurvedic compost was found to be the best one in reducing the blight infection.

**Table 40. Effect of various composts on biometric characters of tomato**

| Treatments | Treatment details | * Plant height (cm) | | * Days to flowering (DAP) | * Days to first harvest (DAP) |
|---|---|---|---|---|---|
| | | 30 DAP | 60 DAP | | |
| $T_1$ | Ayurvedic compost ( Full basal) | $30.25^{cd}$ | $46.64^{abcd}$ | 46.13 | 65.33 |
| $T_2$ | Ayurvedic compost (Two splits ) | $37.74^{abc}$ | $52.02^{abc}$ | 43.66 | 66.00 |
| $T_3$ | Coir pith compost ( Full basal) | $35.83^{abc}$ | $41.16^{cde}$ | 46.00 | 73.00 |
| $T_4$ | Coir pith compost (Two splits ) | $26.33^{d}$ | $39.44^{de}$ | 49.00 | 73.66 |
| $T_5$ | Leaf litter compost ( Full basal) | $31.00^{bcd}$ | $41.60^{bcde}$ | 47.00 | 73.66 |
| $T_6$ | Leaf litter compost (Two splits ) | $30.75^{cd}$ | $39.08^{de}$ | 46.66 | 68.66 |
| $T_7$ | Mixture compost ( Full basal) | $40.25^{ab}$ | $43.08^{bcde}$ | 45.66 | 68.66 |
| $T_8$ | Mixture compost (Two splits ) | $31.73b^{cd}$ | $44.46^{abcde}$ | 44.00 | 76.00 |
| $T_9$ | Aerobic compost ( Full basal) | $42.74^{a}$ | $47.97^{abcd}$ | 45.66 | 70.00 |
| $T_{10}$ | Aerobic compost (Two splits ) | $39.50^{abc}$ | $51.50^{abc}$ | 44.00 | 66.00 |
| $T_{11}$ | Cow dung @50t/ha ( Full basal) | $44.42^{a}$ | $53.25^{ab}$ | 43.66 | 66.00 |
| $T_{12}$ | Cow dung @50t/ha (Two splits ) | $36.96^{abc}$ | $46.26^{abcd}$ | 47.00 | 69.00 |
| $T_{13}$ | Copper hydroxide (2g/l) + NPK | $38.58^{abc}$ | $57.97^{ab}$ | 46.33 | 70.00 |
| $T_{14}$ | NPK as per POP | $43.58^{a}$ | $59.96^{a}$ | 44.66 | 66.66 |
| $T_{15}$ | Absolute control | $23.58^{d}$ | $34.47^{f}$ | 51.66 | 73.66 |
| CD (0.05) | | **7.90** | **10.46** | - | - |

* Mean of three replications

Figures followed by same letter do not differ significantly according to DMRT

## Table 41. Effect of various composts on yield parameters of tomato

| Treatments | Treatment details | * Average number of fruits/plant | * Average weight of fruit (g) | * Yield/ plant (g) | * Yield /6.48 $M^2$ plot (kg) |
|---|---|---|---|---|---|
| $T_1$ | Ayurvedic compost ( Full basal) | $16.33^{abc}$ | $29.21^{bc}$ | $597.39^{abcde}$ | $8.9^{b}$ |
| $T_2$ | Ayurvedic compost (Two splits ) | $16.92^{abc}$ | $40.36^{a}$ | $626.90^{abcd}$ | $8.9^{b}$ |
| $T_3$ | Coir pith compost ( Full basal) | $15.29^{bc}$ | $27.71^{bc}$ | $582.60^{abcde}$ | $5.53^{def}$ |
| $T_4$ | Coir pith compost (Two splits ) | $13.92^{bc}$ | $28.53^{bc}$ | $373.98^{de}$ | $4.27^{f}$ |
| $T_5$ | Leaf litter compost ( Full basal) | $14.49^{bc}$ | $34.12^{ab}$ | $482.55^{bcde}$ | $6.56^{cde}$ |
| $T_6$ | Leaf litter compost (Two splits ) | $15.00^{bc}$ | $29.38^{c}$ | $419.23^{cde}$ | $6.3^{cde}$ |
| $T_7$ | Mixture compost ( Full basal) | $16.75^{abc}$ | $31.65^{abc}$ | $499.83^{bcde}$ | $7.5^{bcd}$ |
| $T_8$ | Mixture compost (Two splits ) | $18.58^{ab}$ | $31.00^{bc}$ | $676.36^{abc}$ | $8.57^{b}$ |
| $T_9$ | Aerobic compost ( Full basal) | $18.49^{ab}$ | $29.25^{bc}$ | $513.19^{bcde}$ | $7.9^{bc}$ |
| $T_{10}$ | Aerobic compost (Two splits ) | $14.00^{bc}$ | $25.91^{bc}$ | $505.80^{bcde}$ | $5.5^{def}$ |
| $T_{11}$ | Cow dung @25t/ha ( Full basal) | $16.92^{abc}$ | $40.15^{a}$ | $574.24^{abcde}$ | $7.2^{bcd}$ |
| $T_{12}$ | Cow dung @25t/ha (Two splits ) | $14.67^{bc}$ | $30.35^{bc}$ | $484.94^{bcde}$ | $6.5^{cde}$ |
| $T_{13}$ | Copper hydroxide @0.2g/l + NPK | $19.97^{a}$ | $34.15^{ab}$ | $799.34^{a}$ | $12.4^{a}$ |
| $T_{14}$ | NPK as per POP | $20.82^{a}$ | $30.03^{bc}$ | $741.57^{ab}$ | $10.9^{a}$ |
| $T_{15}$ | Absolute control | $13.00^{c}$ | $25.22^{bc}$ | $331.86^{e}$ | $4.8^{ef}$ |
| CD (0.05 ) | | **6.06** | **8.23** | **242.1** | **1.78** |

* Mean of three replications

Figures followed by same letter do not differ significantly according to DMRT

# Discussion

# 5. DISCUSSION

Application of high input technologies for the intensification of agriculture and related industries has resulted in manifold increase in generation of various lignocellulosic agrowastes. Human, livestock and crops produce approximately 38 billion metric tons of organic wastes world wide each year and accumulation of wastes, particularly organic wastes, is becoming a serious hazard responsible for deterioration of environment. In India, it is estimated that, 700 millions tons of organic waste is generated annually leading to challenges for its safe and sustainable disposal (Nagavellama *et al.*, 2006; Zeinhom *et al.*, 2010).

Natural biodegradation with soil microbes supports organic waste recycling by converting the wastes into valuable resources of plant nutrients which adds to soil fertility. Composting is a natural process of organic waste management governed by microorganisms. Compost provides nutrients to enhance plant growth, control of soil borne pathogens and helps in bioremediation of toxic soils. Use of chemical fertilizers to exploit high yielding potentiality of crops resulted in the general decline of the soil productivity and health. The problems of imbalance in nutrient availability, soil health deterioration and productivity decline, brought in the awareness to sustain the productivity level. It has become imperative to maintain fertility status which can be achieved by using organic compost.

Organic matter is the main source for recycling of plant nutrients. Cellulose, the major component of plants is the most abundant biopolymer in nature and is a sustainable source of fuel and nutrients. Lignin, being integral cell wall constituent provides strength to plant and resistance to microbial degradation. Likewise, tannins are naturally occurring water soluble polyphenols of varying molecular weight which inhibit the growth of a number of microorganisms, resist microbial attack and are recalcitrant to biodegradation (Field and Lettinga, 1992). Organic matter rich in lignin and phenolic compounds are not easily biodegradable. Composting of ayurvedic medicinal wastes is reported to be rather very difficult and laborious process. Addition of bioinoculants has been reported

to accelerate the composting process of such agrowastes (Preetha, 2003 and Rajan *et al.*, 2005). Earlier reports also showed the potentiality of different microbes in accelerating rapid decomposition of various agrowastes and there by reducing the maturity period of compost. Likewise, partially composted materials using selected microorganisms make vermicomposting faster as compared to normal process (Gaur *et al.*, 1982; Singh and Sharma, 2002).

Composting period often depends on chemical composition of the substrates. Agrowastes such as ayurvedic waste, coconut by products like coir pith and dried coconut leaves, cashew wastes, leaves of teak and mango are not easily biodegradable due to the presence of high amount of lignin, tannin/phenolic compounds. The best alternative lies in evolving rapid methods for composting by adding selective microbial degraders of these materials. Natural composting process which requires long duration also results in loss of nutrients. Hence, the accelerated biodegradation will not only reduce the composting period, but also enhance the quality by increasing nutritive value of composts. The enriched compost, through microbial inoculation is reported to have high nutrient content and there by enhance the crop yield (Singh and Sharma, 2002). Hence, the use of compost will not only stimulate the plant growth, but also suppress soil borne pathogens.

With this back ground, the present study was undertaken to evaluate the combination of microbial degraders to improve the efficacy of composting and to assess optimum period required to produce good quality composts and its effect on plant growth and disease suppression.

Microorganisms are well known for their role in enzymatic degradation and utilisation of complex organic substances such as lignin and tannins. Earlier studies have shown that, increase of microbial population especially the lignocellulosic microorganisms in compost will help in enhancing lignocellulose waste decomposition and hasten the process of composting with different substrates (Beguin and Aubert, 1994; Tengerdy and Szakacs, 2003). Hence, a purposeful attempt was made to isolate various ligno-phenolic degraders from soil

and partially degraded substrates through sampling, enumeration and selection. For this, 53 samples including soils and partially degraded substrates were collected from 15 sources of 34 locations throughout Kerala and from border areas of Tamil Nadu. The isolations were carried out from both enriched and non enriched samples. The enrichment of samples resulted in an increase in the microbial count as compared to non enriched ones. Importance of enrichment in the isolation of lignolytic microbes has been reported by Bandounas *et al.* (2011). Mohan and Ponnusamy (2011) observed high count of bacteria and fungi with the addition of raw materials as carbon sources compared to that without carbon sources.

Isolation of efficient microbial degraders from enriched and non enriched samples from various sources have to be carried out with appropriate dilutions for identifying the most predominant microorganisms among each type of degraders. Hence, an attempt was made to standardise dilution factors for the isolation of cellulose, lignin and tannin degraders from both enriched and non enriched samples. In the present study, dilutions of $10^{-7}$ and $10^{-5}$ were found to be ideal for isolation of cellulose degraders and $10^{-4}$ and $10^{-3}$ for both lignin and tannin degraders from enriched and non enriched samples respectively using selective medium for each degrader. However, Balamurugan *et al.* (2011) could isolate cellulolytic bacteria from enriched soil samples with $10^{-3}$-$10^{-5}$ dilutions only. The perusal of literature on the isolation of microbial degraders showed variations in the dilution factors according to the samples used and type of microorganisms involved. Bandounas *et al.* (2011) could isolate lignolytic bacteria from soils containing decaying organic matter with $10^{-7}$ dilution and this variation in dilution factor might be due to the use of general media for isolation.

For the selection of potential microbial degraders of ligno-phenolic substrates, screening of large number of samples from different sources and locations are necessary. In order to obtain degraders of cellulose, lignin and tannin, the isolations were carried out on selective media with specific chemical nutrients. Dubo's, lignin sulphonate and tannic acid media were used for the

isolation of cellulose, lignin and tannin degraders respectively. Highest population of microbial degraders was observed with partially degraded substrates than the soil samples. Dominance and diversity of degraders in the decomposed substrates can be explained due to the easy availability of nutrients in these samples. The population of different degraders found to be varied with locations and sources which may be due to the preference of microorganisms for their energy sources and other factors such as pH and temperature.

In the present study, ayurvedic substrates, especially the *kashayam* wastes, recorded highest population of cellulose degraders (562 x $10^{-7}$ $cfug^{-1}$). This increased population in the *kashayam* samples may be due to the difference in the ingredients used in the formulation and subsequent preference of degraders for their energy sources.

Samples from coir fibre extraction areas also recorded considerable population of cellulose degraders as compared to coir retting areas. It is interesting to note that, the samples from two coir fibre extraction areas of Kalavoor and Gopalapuram yielded maximum population of 171 x $10^{-7}$ $cfug^{-1}$ and 142 x $10^{-7}$ $cfug^{-1}$ among the samples of coconut by products. The traditional coir retting involves submergence of coconut husks in the saline back waters for a particular period and the anaerobic conditions prevailing during the process along with water salinity may be the limiting factors for the population build up of aerobic microbial degraders in these areas. In contrast, mechanical coir extraction which surpasses this submergence in saline waters might have contributed to the abundance of degraders in these two locations.

In addition, vermicompost, straw spent mushroom dumping sites and termite mound showed appreciable population of cellulose degraders. This is similar to the observations made by Balasundaram (2009) who noticed variation in population of cellulose degrading actinomycetes in different compost samples Gupta *et al.* (2012) recorded eight cellulose degrading bacteria from the gut of different invertebrates including termites.

It is worthwhile to mention that, ayurvedic wastes also recorded high population of both lignin and tannin degraders. The plants and plant parts used in ayurvedic preparations are rich in lignin and phenols and the easy availability of these chemicals from the processed plant parts might have contributed to the luxuriant growth of these degraders.

It is noted that, the samples from coir pith dumping site, Andiyoor, yielded high population of lignin - tannin degraders whereas, population of cellulose degraders were prominent in coir fibre extraction areas. Samples from forest areas, teak plantations and mango orchards recorded high count of lignin - tannin degraders with maximum in the samples from Peechi teak plantations. Undisturbed forest and plantation areas are well known for the diversity of microorganisms and the high population of degraders recorded in the samples of these locations confirmed this fact. As the above studies are unique, there are no supporting evidences to substantiate these findings.

Enumeration of cellulose degraders showed all the three types of microorganisms with dominace of actinomycetes among the total 125 degraders. This is in line with the report of Nurkanto (2010) who isolated seven different genera of cellulose degrading actinomycetes from various soil samples.

With respect to lignin and tannin degraders, fungi were the most predominant one, recording 96 and 99 per cent of the total population. This is well explained by the ubiquitous presence of enzyme system consisting of lignin peroxidase, manganese peroxidase and laccase in fungal degraders (Ball *et al.*, 1989; Eriksson *et al.*, 1990 and Godden *et al.*, 1992).

Next aspect to be discussed is the individual evaluation of isolated microbes for their potential to degrade respective chemical component on their selective media. Of the 125 cellulose degraders, 31 showed diffusion zones more than 4.0cm and another 22 with zones between 3and 4 cm. Of the three types of degraders, fungi was the most effective and the isolates, ACF-4 of ayurvedic waste (6.6 cm), TCF-5 of teak leaves (6.0 cm) and ECF-2 of elephant dung

(5.5 cm) were the most promising ones. Similar to this, Lynd *et al.* (2002) also reported fungi as the well known degraders of cellulosic substrates. In addition, bacterial isolates from fresh cow dung (CdCB-1), coconut by products (CCB-8) and actinomycete, CaCA-3 of cashew litters were also found effective recording diffusion zones more than 4.0 cm. Lu *et al.* (2005) reported *in vitro* efficacy of mesophilic bacteria isolated from flower stalk vegetable waste in utilizing cellulose, recording clear zones ranged from 2.55 - 6.40 cm.

Potentiality of 133 lignin and 120 tannin degraders were tested on their selective media. Of the total lignin degraders, 30 per cent showed clear zones above 4.0 cm and two predominant fungal degraders from coconut by product samples CLF-33 and CLF -14 belonged to *Aspergillus* spp. were the most efficient in lignin degradability. Hence, the fungi other than *Aspergillus* spp. which showed diffusion zones >3 cm were selected in addition to the actinomycete, ALA-1. Mc Carthy and Broda (1984) reported lignolytic activities of *Streptomyces* sp. confirming our result on the lignin degrading ability of actinomyetes. Even though the *Aspergillus* degraders, CTF-25 and CTF-33 showed maximum tannin degradation, the *Penicillium* isolate, CaTF-7 and ATY-1, the yeast isolate were selected for the further study. Observations made by Knudson (1913), Yamada *et al.* (1968) and Saxena *et al.* (1995) who recorded tannin degradation by the genera, *Aspergillus* and *Penicillium* supported the findings of present investigation. Likewise, the role of yeast in tannin degradation was reported by Aoki *et al.* (1976) and Otuk and Deschamps (1983) and this study confirmed their findings.

Generally microorganisms possess well established enzyme system to utilize different energy sources available for their survival. The potentiality of a degrader to utilize different carbon sources is an important prerequisite for including in a consortium for degrading a variety of agrowastes. Cross degradability of various degraders were studied on media other than their selective medium. All the tested degraders produced prominent diffusion zones on other media which clearly indicated their ability to degrade all the three chemical

components since they are not component specific. William and Crawford (1983) noted the ability of fungi to utilize variety of carbon sources which included cellulose and lignin. Thormann *et al.* (2002) and Mukhilis *et al.* (2013) reported the utilization of cellulose and tannic acid by different fungal isolates including *Trichoderma* spp. which are in confirmation with the present study.

The ability of microorganisms to utilize a particular component in media under *in vitro* condition does not necessarily imply the same ability on actual substrates during decomposition. Hence, it has become pertinent to evaluate the efficiency of selected degraders on their respective host substrates. Thirty three selected degraders tested on their host substrates under *in vitro* condition showed composting period of 40 -125 days depending on the degraders and the substrates (Fig 5). Among the 33 degraders tested, 21 showed the lowest composting periods on their respective host substrates with 40, 43, 43 and 60 days for ayurvedic waste, coir pith, elephant dung and leaf litters respectively. Degradative potential of lignocellulolytic microbes have been extensively studied by various workers. Studies conducted by Gaur *et al.* (1982) recorded one month reduction in the composting period of mixed substrate consisting of jowar stalk and wheat (5:3) and jamun leaves with individual inoculation of mesophilic cellulolytic fungi such as *Aspergillus* sp., *Aspergillus niger*, *Trichoderma viride* and *Penicillium* sp. Makkar *et al.* (1994) observed biodegradation of tannin rich oak leaves by *Sporotrichum pulverulentum*. Similarly, Preetha (2003) and Sushama (2005) observed reduction in composting periods of ayurvedic waste and coir pith with the addition of *Pleurotus* sp. which also supported the present results showing the role of microorganisms in the rapid degradation of lignocellulolytic agrowastes.

In order to confirm the *in vitro* efficacy of selected degraders on respective substrates, 22 degraders were further tested for their efficiency under *in vivo* condition recording composting periods of 45, 45, 50, 60 and 65 days for ayurvedic waste, coir pith, elephant dung, cashew and teak leaves respectively (Fig 6). Thus these findings support Preetha (2003) who reported reduction in composting period of ayurvedic waste to 54 days by mushroom fungi, *Pleurotus*

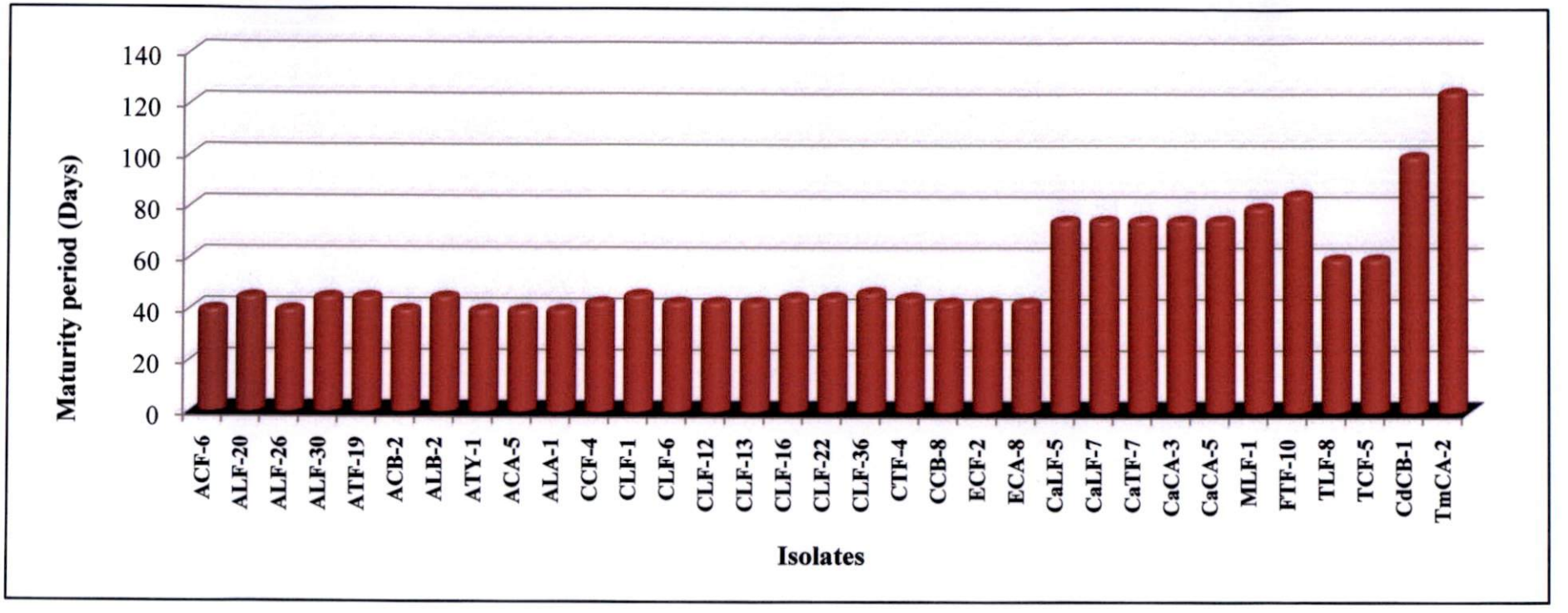



**Fig 5. *In vitro* evaluation of selected degraders on respective host substrates**

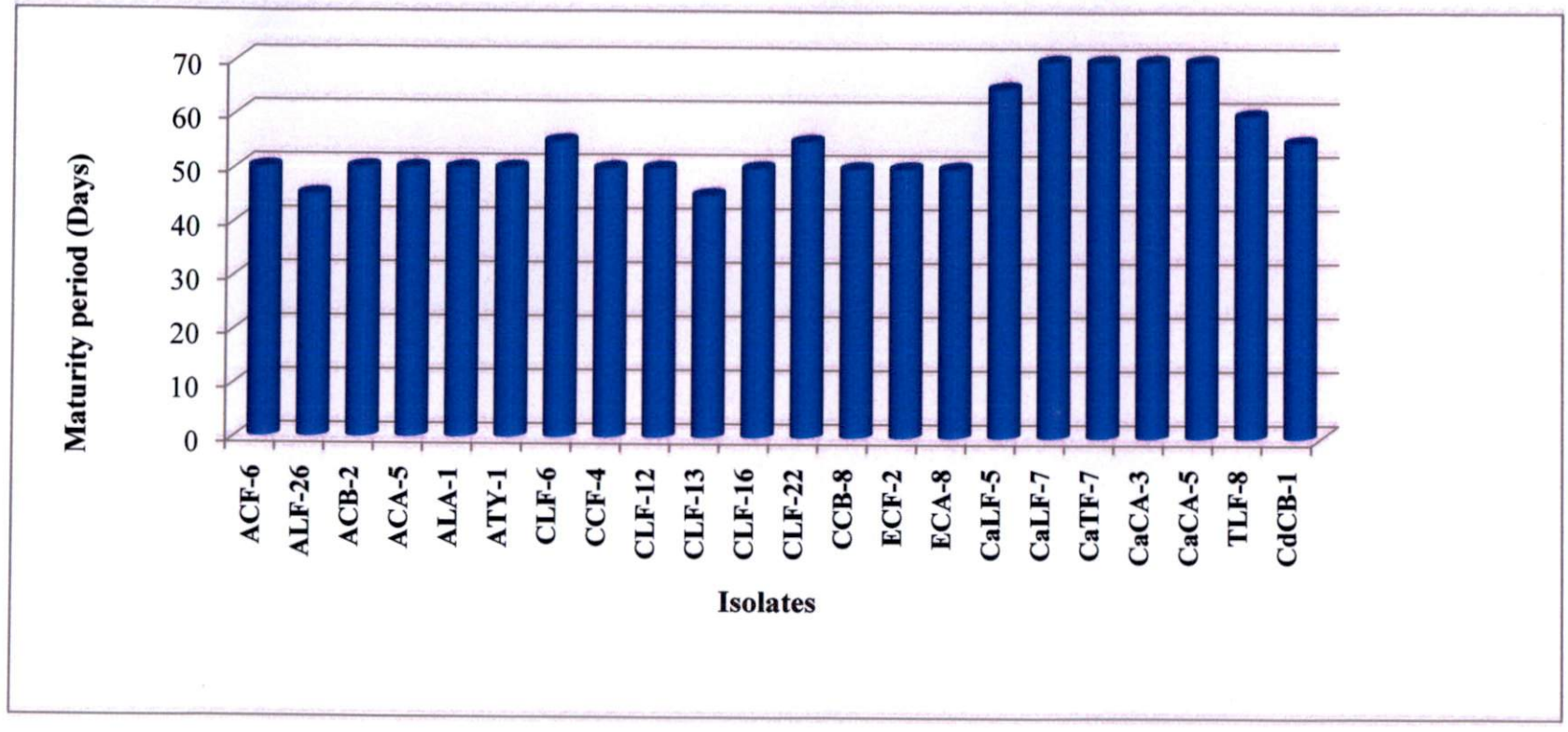



**Fig 6. *In vivo* screening of selected microbial degraders on respective host substrates**

*platypus* and *Schizophyllum commune*. Sushama (2005) also observed reduction in composting period of coir pith to 60 days on composting with *Pleurotus* sp. along with cow dung and goat manure.

During the composting of organic matter, the hydrolysis of polysaccharide constituents by the secreted enzymes would be expected to produce a mixture of carbon compounds. Hence, the ability of selected degraders to utilize a mixture of energy sources simultaneously was studied by inoculating these degraders on lignin - tannin rich plant residues other than their respective host substrates which showed early maturity of 45 -50 days for ayurvedic waste and elephant dung, 45 - 55 days for coir pith and 60 - 70 days for leaf litters. This result indicated that, selected degraders are capable of degrading any lignin –tannin rich agrowastes and are suitable for the microbial consortium. Similar to this, Prasad *et al.* (2014) also achieved degradation of various agrowastes by the bacterial degraders, *Bacillus pumilus* and *Mesorhizobium* sp.

Investigation on development of microbial consortium is focussed on the compatibility of selected microbes with complex metabolic interactions. The synergistic interactions between compatible partners may also contribute to overcome the nutritional limitations in poor agricultural residues (Gutierrez – Correa *et al.*, 1999). The incompatibility of the co-inoculants can also arise because the degraders may inhibit each other. Thus an important prerequisite for the successful development of consortium appears to be the compatibility of co-inoculated microbial degraders. Explorations on mutual compatibility of selected degraders are of great interest, in order to provide the basis for development of microbial consortium. Twenty two degraders including 13 fungi, three bacteria, three actinomycetes and one yeast were tested *in vitro* for their compatibility and all the degraders were found compatible. The interactions between most of the fungal degraders exhibited independent growth without overgrowth. In addition, intermingling or thickening of hyphae at meeting point was also observed. It is also noted that, all bacteria, actinomycetes and yeast were compatible among themselves and also with fungal degraders. None of them

recorded lysis or inhibition at the junctures, confirming their compatibility and suitability to be included in microbial consortium. In the same type of studies, Balasundaram (2009) found the compatibility between fungi (*Aspergillus* spp.), bacteria (*Bacillus* sp.) and actinomycete (*Streptomyces* sp.) selected for microbial consortium for the degradation of ayurvedic herbal waste, coir pith and saw dust. Kausar *et al.* (2010) and Mohammad *et al.* (2011) recorded full or partial mutual intermingling of hyphae or inhibition at contact point recording compatible, partially compatible and incompatible interactions.

Studies were further carried out for the identification of microorganisms selected for consortium. The fungal degraders were identified based on cultural and morphological characters and these characters were compared with the descriptions documented by various workers and also confirmed by NCFT, New Delhi and ARI, Pune. Accordingly, the fungal isolates were identified ECF- 2 as *Trichoderma harzianum* (elephant dung), ALF- 26 as *Paceilomyces variotii* (ayurvedic waste), CaLF-5 as *Penicillium oxalicum* (coir pith), CaTF-7 as *P. chrysogenum* (cashew leaves) and TLF-8 as *Talaromyces* sp. (teak leaves). *Trichoderma* spp. are known to be potent degraders in composting of lignocellulosic materials by producing oxidative and hydrolytic enzymes (Zayed and Abdel-Motaal, 2005; Shafique *et al.*, 2009) and *T. harzianum* as an effective degrader of various lignocellulolytic agrowastes has been reported by Salma and Gunarto (1996); Singh and Sharma (2002); Mukhilis *et al.* (2013). Role of *Paceilomyces* spp. in decomposition of various agrowastes has been reported by Rasal *et al.* (1988). Goyal and Sindhu (2011) also observed effective decomposition of agrowastes by the consortium involving *Paceilomyces* spp. Gaur *et al.*(1982); Mohammad *et al.*(2011) and Kavitha *et al.*(2013) observed *Penicillium citrinum* and *P. chrysogenum* in the degradation of lignocellulolytic agrowastes and reported their saprophytic nature. Suseela and Nandy (1985) noticed tannin decomposition by *P. chrysogenum.* Fernandez *et al.* (2008) reported the cellulase production by *Talaromyces* spp. and the role of its perfect stage, *Penicillium* sp. in biodegradation is very well reviewed. Lignocellulolytic potential of actinomycetes is well documented and the actinomycetes included in

the present study are identified as *Streptomyces albus* and *S. niveus*. Balasundaram (2009) reported the potentiality of four species of *Streptomyces* in the consortium for rapid decomposition of ayurvedic herbal waste, coir pith and saw dust. Mc Carthy and Broda (1984) also noted the lignolytic nature of *Streptomyces* sp. Bacterial isolate from fresh cow dung identified as *Arthrobacter* sp. (CdCB-1) and *Massilia* sp. (CCB-8) from coconut by products *were* selected for the microbial consortium. A vast search on literature revealed that, *Arthrobacter* sp. has been reported as potent actinobacteria for biodegradation of peanut hull lignin (Kerr *et al.*, 1983). Furthermore, there are reports on the use of *Bacillus* sp. isolated from fresh cow dung in decomposition of various agrowastes (Girija *et al.*, 2011). The bacterial isolate, *Massilia* sp. from coconut by products is also found to be an efficient degrader of cellulose. However no other studies have revealed the potentiality of this bacterium as a degrader of agrowastes in the published literature. Perhaps, this may be the first report on the use of this bacterium for the bioconversion of various agrowastes.

Based on the ability to degrade all the three chemical components, type and species of organisms, early maturity under *in vitro* and *in vivo* studies and the mutual compatibility, two consortia were developed and tested under *in vitro* condition, of which (consortium – I) consisted of two nonhuman pathogenic *Aspergillus* spp. (*A. nidulans* and *A. sclerotiorum*) and consortium –II (without *Aspergillus* spp.). Among these, consortium - II was found more effective in reducing the maturity periods and was selected for further investigations. Composting of various substrates using this microbial consortium showed reduced maturity periods of 35 -57 days and 43 -65 days under *in vitro* (Fig 7) and *in vivo* (Fig 8) conditions.

Further, the degrading potentiality of microbial consortium was assessed with large quantity of substrates along with cow dung slurry alone and in combination with consortium as comparison (Fig 9). Among the three treatments, early maturity was observed with consortial application and the composting

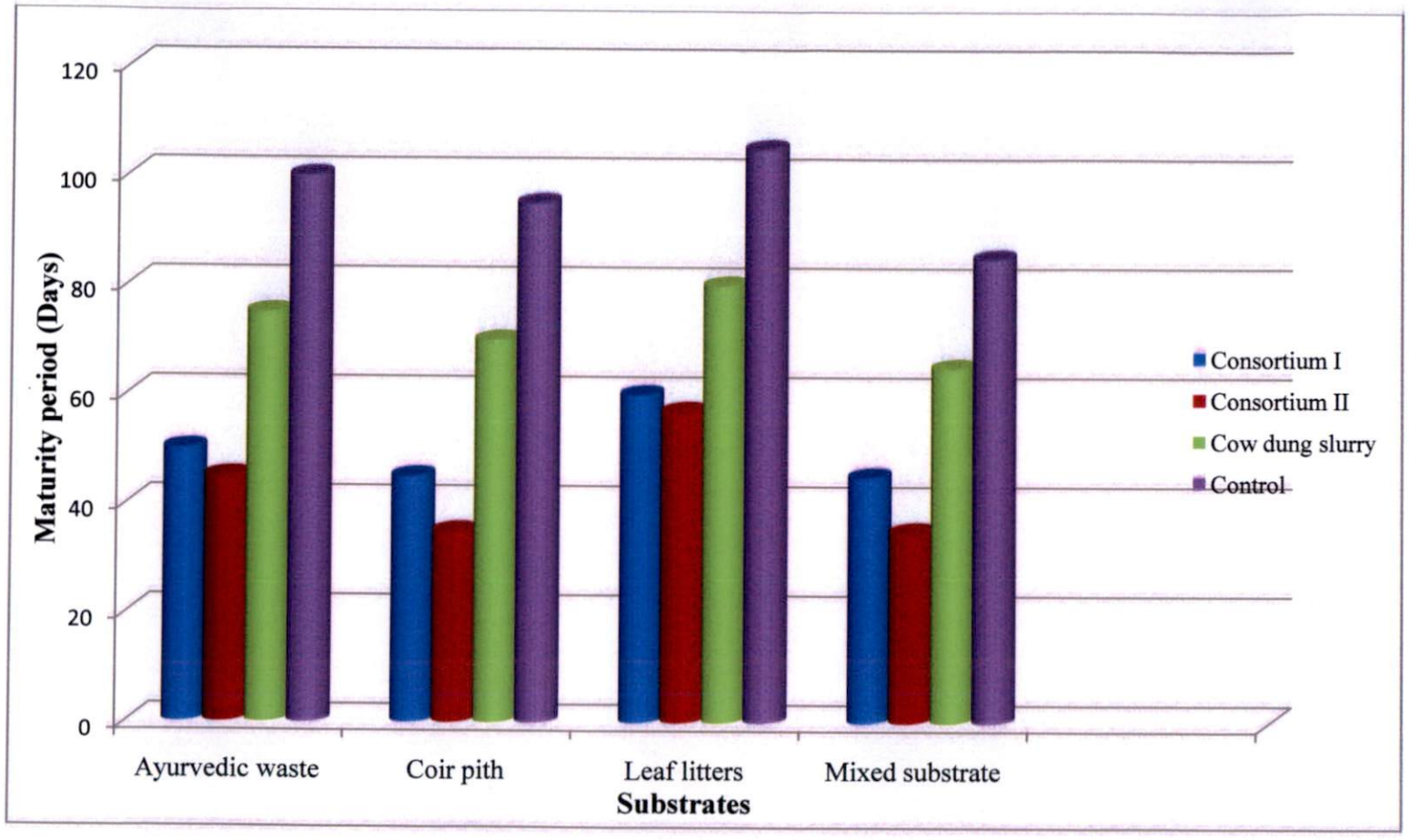



**Fig.7. *In vitro* screening of microbial consortium on various substrates**

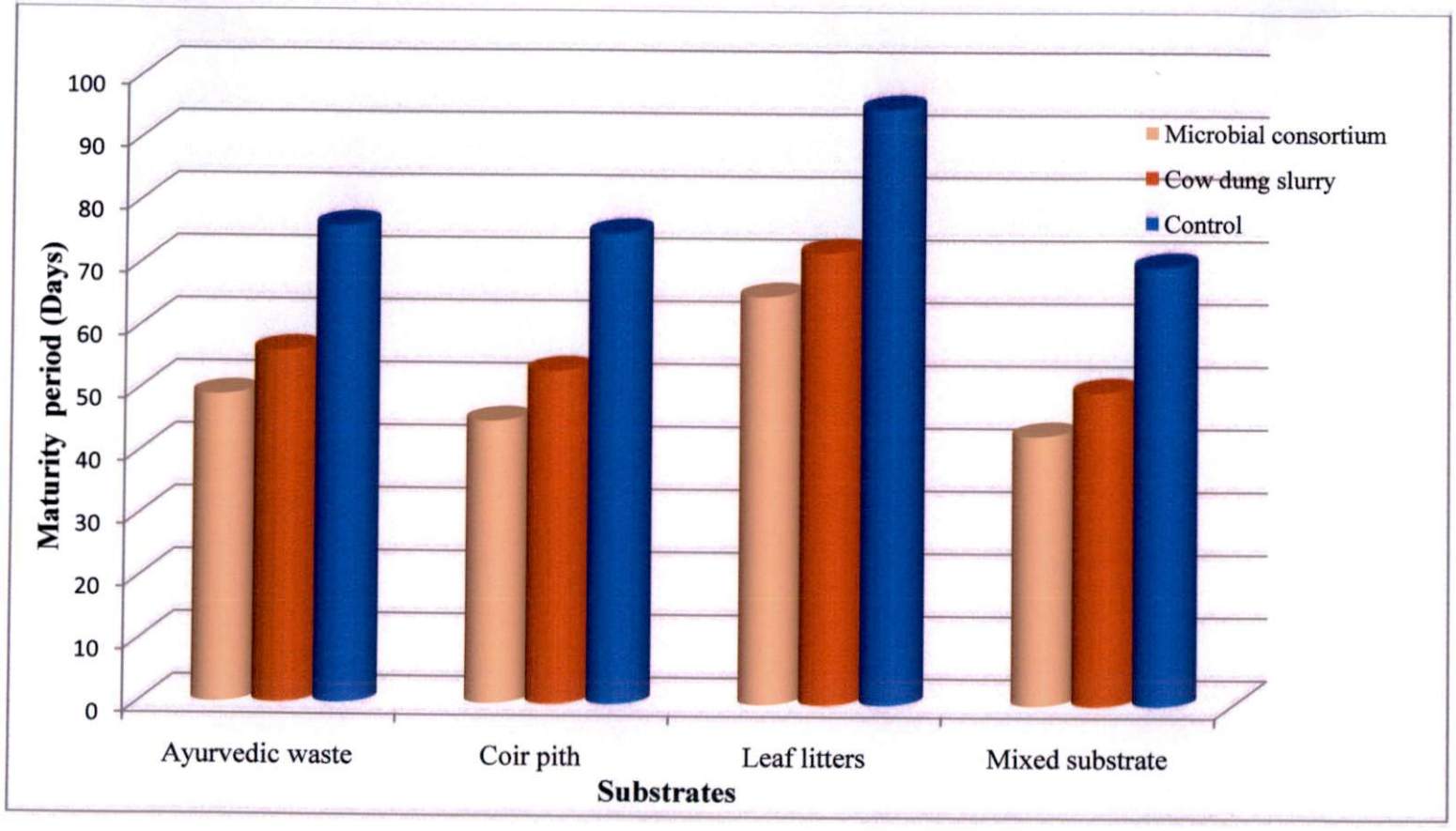
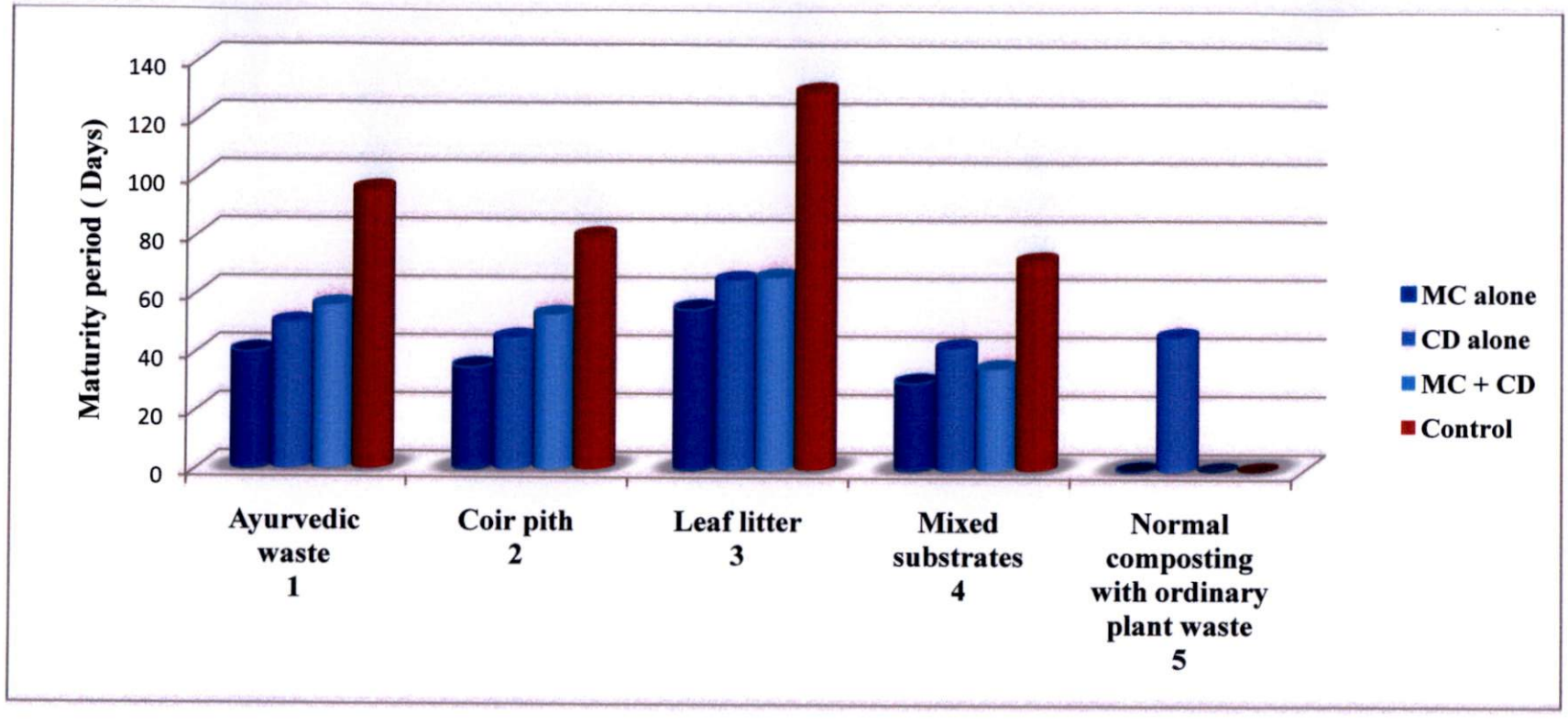



**Fig 8. *In vivo* screening of microbial consortium on various substrates**




MC- Microbial consortium alone, CD alone- Cow dung slurry alone, MC+ CD- Consortium + Cow dung

**Fig 9. Large scale composting of ligno-phenolic substrates**

period found to be varied with substrates. The inoculation of substrates with 20 per cent microbial consortium alone recorded early maturity of all substrates with 30, 35, 40 and 55 days against 42, 45, 50 and 65 days in cow dung slurry treatment for mixed substrates, coir pith, ayurvedic waste and leaf litters respectively.

The role of microorganisms in reducing the composting period of lignocellulosic substrates has been studied by various workers. Sushama (2005) noticed reduction in the composting period of coir pith from 120 to 60 days with *Pleurotus* sp. Vermicomposting of ayurvedic waste pretreated with lignin degrading mushroom fungi, *Pleurotus platypus/ Schizophyllum commune* recorded 54 days for maturity against 110 days in normal composting (Preetha, 2003) whereas, Balasundaram (2009) observed much reduction in the composting period with 22 days for ayurvedic herbal waste and 26 days for coir pith using microbial consortium consisting of 14 cultures which might be due to the increased number of microbial degraders, low quantity of the substrates used and also due to the inclusion of two *Aspergillus* spp. as *Aspergillus* spp. are reported to be the most efficient degraders of various agrowastes. These reports support the findings of the present investigation on the effect of microbes in reducing the composting periods of various agrowastes. However, Naik (2007); Mohan and Ponnusamy (2011) and Kavitha *et al.* (2013) observed longer composting periods of 90 days for grape vine residues, 60 days for sugar cane trash and 90 days for oil palm empty fruit bunch.

It is also necessary to understand the role of temperature in the decomposition of organic matter, since it is the primary factor which controls microbial community succession during composting. A typical composting process proceeds through a series of phases including a rapid temperature increase, sustained high temperature and a gradual cooling of the composting mass. Keeping view of the aforementioned facts, daily temperature variations of the composting piles were monitored and recorded. Temperature variations recorded, followed a similar pattern irrespective of the treatments and substrates

and the common trend in fluctuation of temperature tend to specify the three stages of composting – mesophilic, thermophilic and cooling and maturation. However, the temperature range in each phase varied according to substrate composition and rate of composting. Temperature of substrates in different treatments varied between 32.1 - 48.4°C on the initial day of composting and gradually increased to 35.7 - 59.2°C on 6 - 13$^{th}$ day. The maximum peak temperatures were observed in the substrates treated with microbial consortium and highest temperature was recorded in ayurvedic waste (59.2 °C) on 13$^{th}$ day followed by mixed substrate (57.2°C) on 9$^{th}$ day of inoculation. The high temperatures recorded with these substrates may be due to the high phenol content in the ayurvedic waste. According to Hashimoto *et al.* (1981) and Kimchie *et al.* (1988), the thermophilic temperatures resulted in rapid degradation of organic matter. Gowda (1996) reported that, during biodegradation, the microbes consume more oxygen to break down organic compounds and the release of heat energy by the microbes during decomposition of organic matter for rapid multiplication resulted in the rise of temperature and faster decomposition of organic matter. The high temperatures noticed in ayurvedic wastes and mixed substrates during the present investigation might have contributed to the rapid decomposition of these ligno-phenolic substrates which are otherwise difficult to degrade by natural means. Gaur (1996) suggested that, the exothermic energy released during composting process generated substantial amount of heat, thus raising the heap temperature and create favourable conditions for thermophilic microorganisms which also confirmed the present findings.

Maintenance of high temperatures within the compost heap for more than three consecutive days is found to be effective for the destruction of pathogens and weed seeds within the heap (Rynk *et al.*, 1992). The temperature peaks recorded in the present study suggests, the process would ensure safe end product for soil application. The thermophilic phase later showed gradual decline and the cooling and maturation phase initiated with decreasing pattern of temperature. Lowest temperature in the compost piles ranged from 26.5 - 34.9 °C in various composts treated with microbial consortium. This is in accordance with the

findings of Balasundaram (2009) and Singh and Saxena (2011) who observed temperature ranges of 30 - 35°C at maturity of composting with microbial consortium.

Various substrates having acidic and neutral pH were found to increase to alkaline range of 7.6 -8.6 after composting in all treatments. It is interesting to note that, ayurvedic waste with a pH 5.3 and 6.6 showed drastic increase to 8.5 and 8.6 on composting with microbial consortium. These findings indicated that, all compost products were found to have alkaline pH on composting, especially those treated with microorganisms and these compost products are good ameliorants for the acidic soils of Kerala. Earlier studies have identified that, microbial activity enhanced the likelihood of achieving a pH range of 5.5 -9.0. This increase in pH of final composts is the result of microbial decomposition of organic acids and subsequent release of ammonia by volatalisation as suggested by Mc Kinley and Vestal (1985). Hike in pH during microbial composting was observed by Arunachalam and Rajasekharan (2009) and recorded pH of 9.6 in the coir pith composted with cyanobacteria and Mini *et al.* (2005) observed pH of 8.9 for vermicomposted cashew leaf litters.

Combination treatment with consortium and cow dung slurry was found to be less effective than cow dung slurry alone with longer composting periods of 36, 40, 51 and 66 days which can be due to the incompatibility of microbes in the consortium and cow dung. Hence, a compatibility study was conducted with the cultures of consortium and cow dung slurry to confirm this aspect, which showed the inhibition of *Penicillium* spp. by the microbes in cow dung. Likewise, *P. variotii*, *S. niveus* and *Massilia* sp. were found to inhibit the microbes in the cow dung indicating the incompatibility of certain degraders of the consortium with cow dung microbes which clearly supported the above findings. Similarly, Goyal and Sindhu (2011) reported longer composting periods in the combination of consortium and cattle dung compared to individual treatments.

No attempts have been made so far in Kerala with respect to the study on potentiality of microbial degraders from agrowastes like ayurvedic waste, coconut by products, elephant dung and leaf litters of forests and plantations areas in the rapid decomposition of ligno-phenolic plant residues. Hence, this is a pilot and detailed study on management of these lignin-tannin rich agrowastes and the study have provided valuable information on the role of microbial degraders for accelerating the rapid degradation of these agrowastes.

Several indices have been employed to assess the stages of composting including colour, odour, temperature and C:N ratio (Zmora - Nahum *et al.*, 2005). Carbon and nitrogen are the most important elements in biodegradation process, as one or other is normally a limiting factor (Richard, 2008). C: N ratio is reported to be a reliable indicator and can be used as an index of compost maturity (Inbar *et al.*, 1990; Bernal *et al.*, 1998). Hence, it was obvious to analyse the C:N ratios of various substrates as well as composts used in the present investigation. The C:N ratio of various substrates used in this study was comparitvely high and varied between 39.3 - 165.7:1 with a maximum (165.7:1) in coir pith. According to Krishnamurthy (1978), the satisfactory initial C:N ratio for composting is 30 - 35 :1 and the composting will be prolonged if the C:N ratio is wider one. At maturity, the C:N ratios of substrates treated with microbial consortium, cow dung slurry and combination of both showed reduction from the initial values. Drastic reduction in C:N ratios was observed in the substrates treated with consortium and maximum reduction from 57.7:1 to 10.4:1 was noticed in ayurvedic compost followed by mixture compost which reduced to 17.5:1 from the initial, 53.5:1. Thus 81 - 47 per cent reduction in C:N ratio is being estimated. The combination of consortium with cow dung slurry and cow dung slurry alone also showed considerable reduction in the C:N ratio with maximum in ayurvedic waste and the corresponding per cent reduction in C:N ratio is worked out to be 76 and 67 per cent respectively.

This reduction of C:N ratio in the final composts can be correlated to the assimilation of substrate carbon to nitrogen for the cell building of microbes involved in the decomposition of organic matter and this suggests clear evidence for the role of microbes in biodegradation of organic matter and subsequent lowering of C:N ratio. Involvement of microbes in decomposition of organic matter and reduction in C:N ratio has been investigated by various workers. Similar results have been observed by Balasundaram (2009) with reduction in the C:N ratio of ayurvedic waste from 35:1 to 10.3:1 on composting with microbial consortium. Preetha (2003) observed reduction in C:N ratio from 32.9:1 to 11.3:1 in the vermicomposted ayurvedic waste pretreated with mushroom fungi. However, in the present study, per cent reduction in C:N ratio was higher as compared to these reports and these reports also confirmed our findings.

In the present study, the C:N ratio of coir pith was comparatively high showing 33.2:1 to 53.67:1 in various treatments. It is to be considered that, the raw materials used for composting had a high C:N ratio of 165.7:1and even though the C:N ratio recorded was comparatively high (53.7:1), it has shown one third reduction, which is in line with the findings of Sushama (2005) who also observed one third reduction in the C:N ratio of coir pith from 75:1 to 25 :1 on composting with *Pleurotus* spp. The high C:N ratio of coir pith compost can also be explained by the presence of unutilized complex nitrogenous substrates (Saidi *et al.*, 2008; Fourti *et al.*, 2011). Furthermore, according to Haider (1992) lignin to organic nitrogen ratio controls carbon mineralisation in plants.

Management of composting process must consider the potential agronomic value of the end product and its suitability to crop plants. Thus it became necessary to analyse the nutrient status of substrates before and after composting with different treatments. A noticeable increase in primary nutrients was observed irrespective of the treatments. However, maximum increase was recorded in the substrates treated with microbial consortium. The nutrient contents of various composts on treatment with microbial consortium ranged from 0.98 - 4.80 per cent for N, 0.21 -0.46 for P and 1.2 -1.60 per cent for K respectively. Among the

composts, highest contents of nitrogen and phosphorus were observed in ayurvedic compost (4.80 % & 0.46 %) whereas, potassium content was found to be maximum in coir pith compost (1.60 %).This increase in the nutrient values of final composts treated with microbial consortium can be due to the proliferation of inoculated microbes and consequent improvement in the degradation of substrates resulting in mineralisation. Earlier workers extensively studied the nutrient status of various composts and Balasundaram (2009) recorded nitrogen contents of 3.57 per cent and 0.64 per cent in ayurvedic herbal and coir pith composts treated with microbial consortium. Whereas, Preetha (2003) noted 3.27 per cent nitrogen in the vermicomposted ayurvedic compost pre-treated with mushroom fungi. Sushama (2005) also recorded increase in the primary nutrient contents of coir pith composted with green leaves and *Pleurotus* spp. The N, P, K contents of leaf litters in the present study was 1.96, 0.42 and 1.16 per cent respectively and Mini *et al.* (2005) observed N, P, K contents of 1.69, 0.44 and 0.58 per cent in vermicomposted cashew apple residues and leaf litters. Thus the present findings confirmed their results. The chemical composition of composts varies depending on the substrates from which it is prepared can tender satisfactory explanation to the variation in the primary nutrient contents of different composts. Thus present study revealed that, rapid degradation of agrowastes, will improve the quality of composts.

Like macronutrients, micronutrients status of composts products is also important, as it is essential for many vital functions of the plant system. Hence, the estimation of micronutrient contents of the composts prepared with microbial consortium was attempted even though, N, P, K contents of different composts were found satisfactory. On estimation, the composts recorded presence of calcium, magnesium, copper, manganese, zinc and iron in varying concentrations. This is in harmony with Kishore *et al.* (2010) and Kumar and Ganesh (2012) who also recorded the presence of various micronutrients such as Ca, Mg, Mn, Zn, Cu and Fe in the final composts treated with fungal degraders.

Presence of heavy metals in finished composts is the main reason for the adverse effects on animal and human health, transmitted through food chain from soil, ground water and plants (Senesi *et al.*, 1999). Consequently analysing the heavy metal contamination in final composts has become important in risk assessment. The analysis for heavy metals carried out in various composts prepared with microbial consortium, did not record the presence of heavy metals except lead. In addition, traces of chromium and nickel were also noticed in ayurvedic compost and the concentration of these elements was much lower than the permissible limits. The perusal of literature did not yield any references on heavy metal contamination in composts produced from organic wastes, even though there are reports on bioremediation of municipal solid wastes by composting with *Phanerochaete chrysosporium* (Liu *et al.*, 2009). This study ruled out the chances of heavy metal contamination and confirmed that, these compost products are safe for agricultural uses.

Next point to be discussed is the biochemical changes occurring during the composting of organic residues with microbial consortium. The biochemical analysis of cellulose, lignin and tannin contents of substrates and final composts treated with consortium was conducted. The results stated that, composting with microbial consortium recorded tremendous reduction in the cellulose, lignin and tannin contents of all substrates (Fig 10, 11, 12 and 13). However, the reduction was found maximum in ayurvedic compost for cellulose (49.38 %), coir pith for lignin (49.02 %) and leaf litters for tannin (98.95 %). This study proved the theoretical expectations and practical experiences of lignocellulolytic microbes producing various enzymes which accounted for the reduction of cellulose, lignin and tannin contents of organic residues during decomposition. Role of microorganisms in the enzymatic degradation of cellulose and lignin contents were studied by various researchers. Preetha (2003) noticed 47.33 and 25.59 per cent reduction of cellulose and lignin in vermicomposted ayurvedic waste.

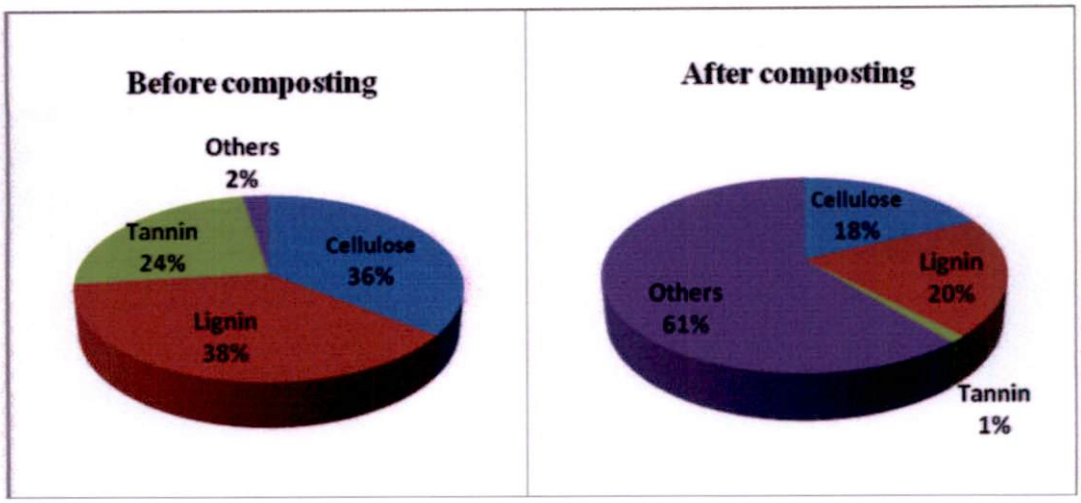



**Fig 10. Biochemical changes in the composition of ayurvedic waste by composting with microbial consortium**

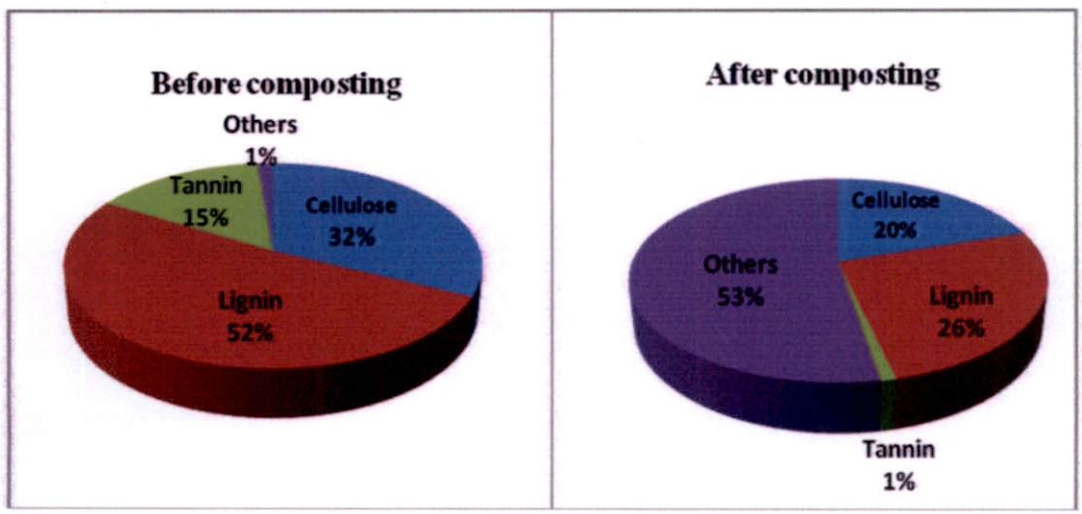



**Fig 11. Biochemical changes in the composition of coir pith by composting with microbial consortium**

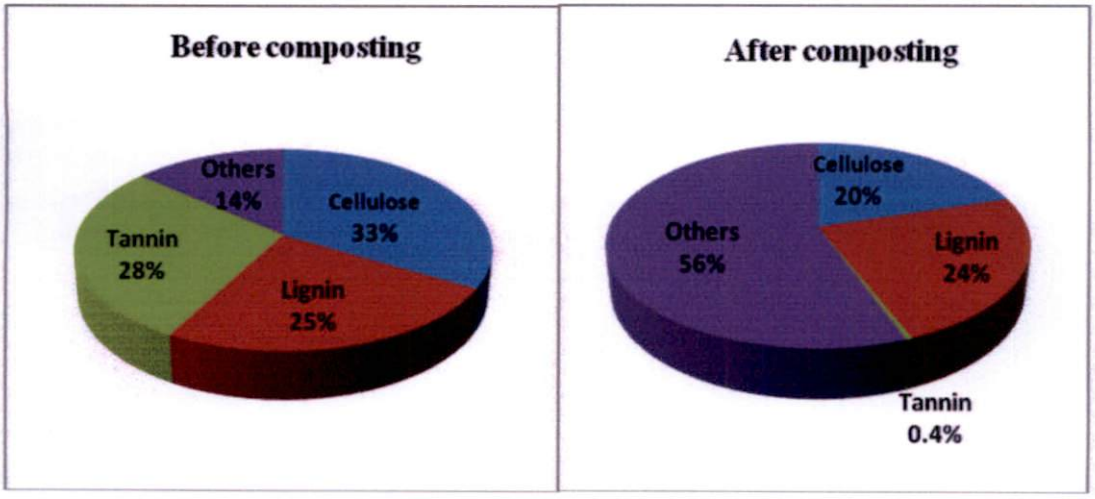



**Fig 12. Biochemical changes in the composition of leaf litters by composting with microbial consortium**

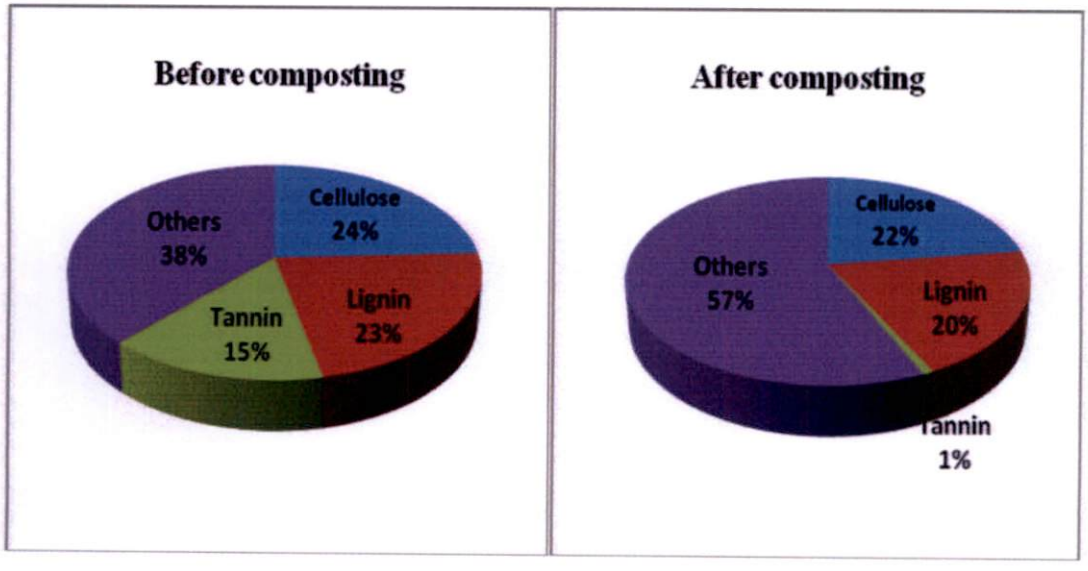



**Fig 13. Biochemical changes in the composition of mixed substrate by composting with microbial consortium**

Likewise, Sushama (2005) and Reghuvaran and Ravindranath (2013) also recorded 43 and 38 per cent reduction in the lignin content of coir pith composted with *Pleurotus* spp. thus, their results confirmed the present findings. The literature review did not reveal much studies on tannin biodegradation, however, Bhat *et al.* (1997) and Crus Hernanadez *et al.* (2005) reported degradation of tannin by *Aspergillus* spp.

Another experiment was undertaken for the large scale composting with talc based consortial formulation @ 1kg/ 100 kg substrate, during two seasons, which also showed reduction in maturity period recording 40,40,45 and 60 days during April- May against 32, 34,34 and 57 days during October – November for mixed substrate, coir pith, ayurvedic waste and leaf litters respectively. This variation in the reduction of maturity periods observed in October – November may be due to high retention of moisture in the substrates during the rainy season. It also showed much reduction in the compost weight in summer, as compared to rainy season. The increase in the weight of mature compost in rainy season may be attributed to the high moisture content prevailing during the season. Thus, the study also indicated that, talc formulation is also equally effective as liquid formulation which is cost effective and easy to operate.

*In toto*, after reviewing the nutrient status and biochemical aspects, it was found that, the composts prepared with microbial consortium can supply all the essential macro and micronutrients to enhance crop productivity. Furthermore, it is also noted that, the final compost products were having low contents of cellulose, lignin and tannin and were of high quality that suitable for highly succulent leafy vegetables.

The survival capacity of the inoculated microbial degraders in mature composts was confirmed by their reisolation on respective media. The results revealed that, all the degraders were viable throughout the composting process by recording viable colonies on their respective selective media. The reisolation also indicated that, the degraders were able to withstand different temperature phases

recorded during the composting process and there are no attempts on reisolation of inoculated microbial degraders in the literature.

For a better understanding of the thermophilic nature of consortial microbes, it was found necessary to study their thermotolerance potential, since the composting process proceeds through various temperature regimes in the mesophilic, thermophilic, cooling and maturation phases. In this study, all the selected degraders remained viable upto temperatures of 60°C. This confirms the results obtained during the reisolation of degraders from mature composts, which also recorded viable colonies of degraders on their selective medium. The thermophilic nature of degraders involved in lignocellulosic bioconversion is of prime importance, since 70 per cent of lignin biodegradation occurs during initial period of composting, when the temperature is maintained at 50°C (Tomati *et al.*, 1995; Horwarth and Elliott, 1996). Recalling back the temperature variations noted during the composting, in which various compost piles recorded peaks upto 59.2 °C and the studies on thermotolerance and reisolation of inoculated microbes revealed that, all degraders included in the consortium are highly thermophilic with good degradation potential. Thermophilic nature of different degraders such as *Paecilomyces* sp. and *Talaromyces* sp. (Dix and Webster, 1995) and *Streptomyces* spp. (Waksman *et al.*, 1939) included in the consortium have been already reported which are in line with the present findings.

Microorganisms are very important for the enzymatic degradation of complex organic materials to nutrients. Lignocellulosic materials are degraded by the concerted action of many lignocellulolytic enzymes and it is presumed that, higher the lignocellulolytic potential higher will be the decomposition of lignocellulolytic wastes. For having basic information on the enzymatic potential of the microorganisms selected for the consortium, a study was conducted for the potential production of different lignocellulolytic enzymes involved in the decomposition of organic matter. During the enzymatic studies, all degraders exhibited activities of cellulase, amylase, xylanase, protease, catalase, lignin peroxidase and polyphenol oxidase in varying proportions. Even though, the study

included different enzymes involved in bioconversions, more thrust was given to three enzymes *viz.* cellulase, lignin peroxidase and polyphenol oxidase which are the key enzymes involved in the degradation of cellulose, lignin and tannins. The activity of cellulase was exhibited by all degraders and maximum was observed in *T. harzianum*. Cellulase production by different *Trichoderma* spp. is noted by Shafique *et al.* (2009) and cellulolytic enzyme production by *T. harzianum* and *T. koningii* has also been reported by Mukhlis *et al.* (2013) and their findings are in accordance with these results. All fungal degraders, one bacterium and an actinomycete showed lignin peroxidase activity. None of the degraders, other than *Penicillium* spp. (Vijaya and Singaracharya, 2005) used in this study is reported to have lignin peroxidase activity. However, Rajan *et al.* (2005) observed lignin peroxidase activity for *P. chrysosporium* and *Pseudomonas putida* employed in coir retting. The activity of poly phenol oxidase was observed by all degraders except *T. harzianum,* bacterial degraders and *Streptomyces niveus.* Mukhilis *et al.* (2013) noted polyphenol oxidase activity by *T. harzianum*, however, in the present study, the fungus did not show the activity of this enzyme. Thormann *et al.* (2002) noted the activity of poly phenol oxidase by the fungi, *Oidiodendron* spp. during *in vitro* trials on the decomposition of tannic acid.

Microbial degraders selected for the development of consortium belonged to different genera of fungi, bacteria and actinomycetes well known for their biocontrol potential. Hence, a study was undertaken to establish the direct antagonistic potential of these degraders against important soil borne pathogens. It is observed that, all the selected degraders showed direct antagonism against most of the test pathogens and *T. harzianum* which showed maximum inhibition of 62.78 per cent against *Phytophthora* sp. was the most competent. Other prominent fungal degraders, *Paceilomyces* sp. and *Penicillium* spp. could also effectively inhibit all tested pathogens. The antagonistic activity of compost inhabiting microbes like, *Trichoderma* spp. (Hoitink and Boehm, 1999; Pugliese *et al.*, 2008) *Penicillium* spp. (Hadar and Gorodecki, 1991) and various actinomycetes (Hardy and Sivasithamparam, 1991) is already reviewed. However, the literature search could not provide any citations on the direct inhibitory activity of such microbial

degraders against soil borne pathogens and this may be the first attempt to elucidate this aspect.

Use of agricultural chemicals is the most widely used strategy for controlling pest and diseases. Though chemicals offer certain degree of protection against the pathogens, chemical control has its own constraints such as cost factor, pollution, non target effect and development of resistance to chemicals. So there is considerable interest in manipulating soil environment to achieve biocontrol of important soil borne pathogens. Moreover, the current trend of zero market tolerance for pesticide residues in agricultural produce resulted in the search of alternate safe strategies in disease control. During recent years application of bioagents as well as soil amendments are found to be effective methods in combating plant diseases. Composts have been used for centuries to maintain soil fertility and crop health and considerable research have been conducted on the disease suppression by compost products (Hoitink and Fahy, 1986; Schuler *et al.*, 1983). The disease suppression is mainly due to the direct interactions between antagonistic microorganisms in the composts and the pathogens by the way of competition, antibiosis or hyperparasitism (Hoitink *et al.*, 1993) and also due to the indirect action by the activation of induced systemic resistance or by the enhancement of plant growth (Zhang *et al.*, 1996; Sang *et al.*, 2010 and Yogev *et al.*, 2010). Keeping these facts in mind, a study was conducted to evaluate the bioefficacy of various ligno-phenolic compost products in the management of two important soil borne diseases of vegetables in Kerala *viz.* bacterial wilt of tomato caused by *Ralstonia solanacearum* and leaf blight of amaranth by *Rhizoctonia solani.*

The efficacy of ligno-phenolic composts products were evaluated against leaf blight of amaranth causing severe crop losses in amaranth especially during rainy season. In the present investigation, even though none of the treatments could give complete protection against leaf blight disease, the severity of the disease could be reduced by the application of composts products especially after the first harvest (Fig 14). Among the treatments, ayurvedic compost application

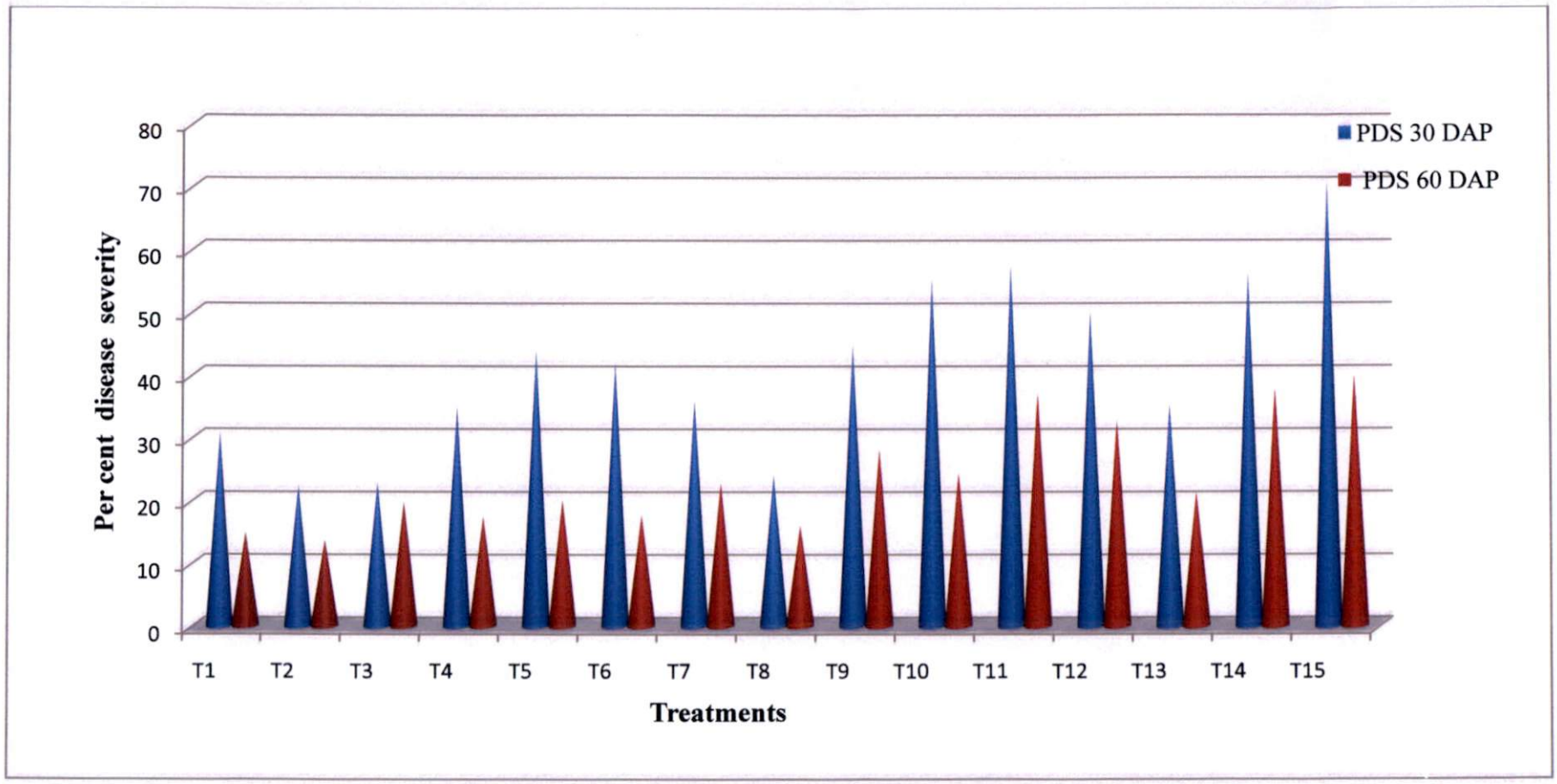



**Fig 14. Efficacy of various composts on the management of leaf blight of amaranth**

was the most effective recording 65.88 - 62.87 per cent reduction over control. In addition mixture, coir pith and leaf litter composts were equally effective in reducing leaf blight infection to 60.17 -50.69 per cent. It is also interesting to note that, the split application was found better than the full basal application with respect to disease severity and yield. Many researchers have observed the effect of compost products in the management of *R. solani* (Krause *et al.*, 2001; Diab *et al.*, 2003 and Scheuerell *et al.*, 2005). Sathianarayanan and Khan (2008) observed *in vitro* suppression of *R. solani* with the extracts of composted and vermicomposted coir pith. Similar to this, Sudha and Lakshmanan (2011) also reported suppression of *R. solani* causing rice sheath blight with the application of coir pith composted with fungal degrader, *Lentinus connatus*. Thus the present findings confirmed the results of above researchers.

With respect to yield, full basal application of cow dung recorded the highest yield of 5.10 kg/5m$^2$ followed by the split application of ayurvedic compost with 4.33 kg/5m$^2$.From the field experiments on bacterial wilt of tomato, it was observed that, all treatments were superior to control with respect to disease incidence and biometric characters. In the present study, soil drenching of copper hydroxide (2g/l) used as comparison check recorded minimum disease incidence of 6.93 per cent at 60 days after planting and it is well known phenomena that, chemicals provide better management than organics. However, the present study showed that, organic products also had an effect on the management of bacterial wilt which varied with different treatments. Among the composts, lowest incidence (13.88 %) was observed for ayurvedic compost which provided per cent reduction over control followed by leaf litter and coir pith composts with 63.36 and 60.02 per cent disease reduction (Fig 15).

It was also noted that, all compost treatments were better than cow dung and chemical fertilizers in checking the disease. Much difference was not observed between full basal and split applications. Split application of ayurvedic compost recorded lowest incidence whereas, full basal application was found effective for coir pith and leaf litters. A search through relevant literature did not

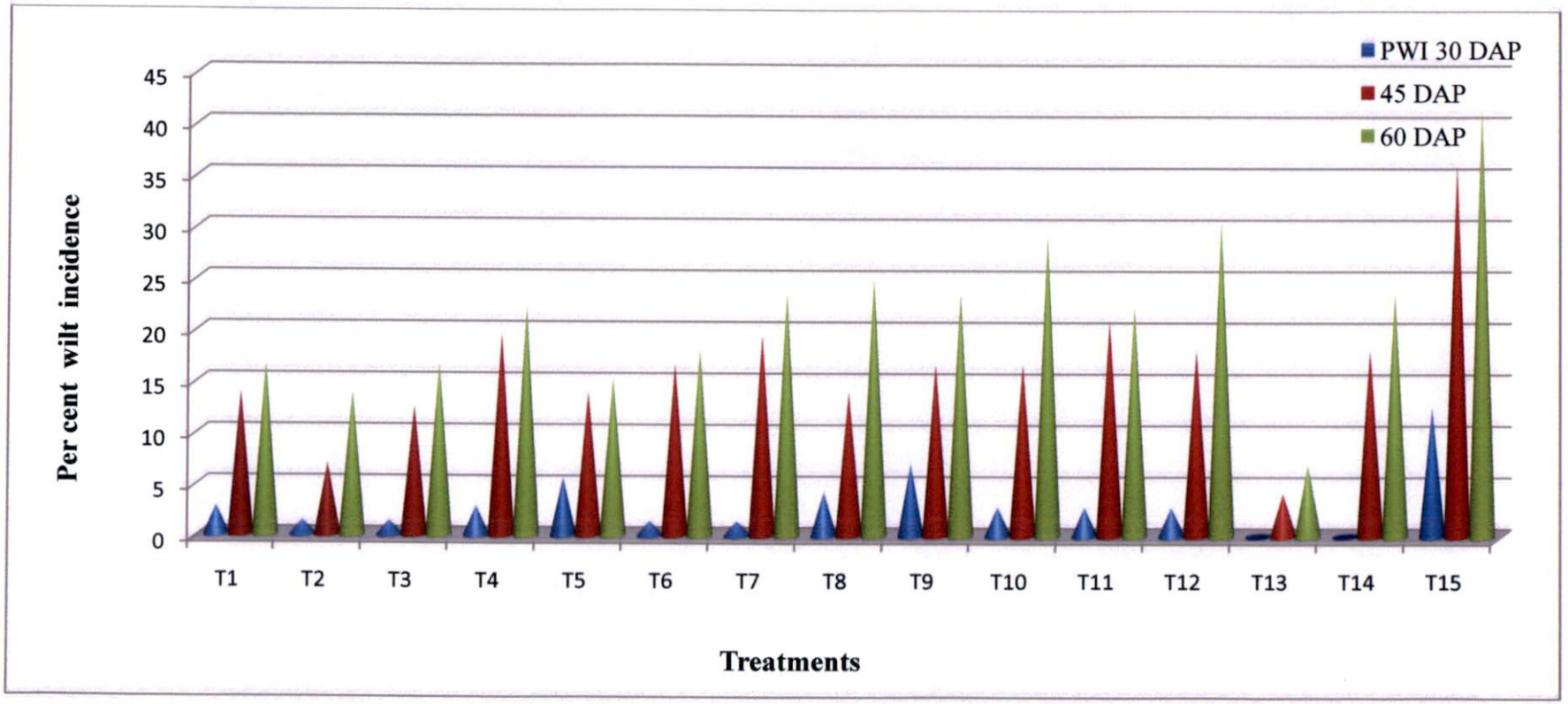



**Fig 15 . Efficacy of various composts on the management of bacterial wilt of tomato**

give any information on the effect of ayurvedic compost in the management of bacterial wilt. However, the suppression of bacterial wilt disease by coir pith compost and coco peat has been reported by Mathew (2004) and Yadessa *et al.* (2010). Mc Keller and Nelson (2003) reported the efficacy of composted leaf litters of deciduous trees for the management of cotton damping off and these reports supported the findings of present investigation.

The effectiveness of a disease management strategy will be complete, when it coincides with the increase in crop yield. As a matter of fact, inorganic fertilizers contribute maximum to the enhancement of crop yield and in the present study also, NPK application along with copper hydroxide recorded maximum yield (12.4kg/6.48 $m^2$) in addition to maximum reduction in wilt incidence, which is followed by application of chemical fertilizers alone (10.9kg/6.48 $m^2$). Composts treatments were also found equally effective with respect to yield. Among the composts, both split and basal application of ayurvedic compost recorded highest yield (8.9 kg/6.48 $m^2$) followed by split application of mixture compost (8.6 kg/6.48 $m^2$). Lazcano *et al.* (2009) observed improvement in plant growth by the addition of composts in tomato plants. The increase in yield of maize and lettuce crops was found to be correlated with the nutrient contents of composts applied (Mrabet *et al.*, 2012) and these reports are in confirmation with the present results.

Considering the overall performances of various treatments, lignin-tannin rich compost products were found effective in suppressing both the soil borne diseases and ayurvedic compost was found to be the best in disease management and in promoting plant growth characters. This can be correlated with the direct and indirect action of composts products which might have resulted in disease suppression and subsequent yield enhancement. The inhibitory effect of extracts of medicinal plants on plant pathogens has been reported by many workers. Mathew *et al.* (2004) observed the inhibitory effect of 10 per cent Adathoda extract on *R. solanacearum* causing bacterial wilt in solanaceous vegetables. However, search on literature has not provided any information regarding the

effect of ayurvedic compost products in the suppression of phytopathogens. Perhaps, this may be the first attempt in the management of soil borne diseases with ayurvedic compost.

The efficacy of compost products in the suppression of plant pathogens may be due to the antagonistic activity of microbial degraders in the compost. Reading back the results of *in vitro* antagonistic activity of selected degraders against soil borne pathogens, it was evident that, all degraders showed inhibitory effect of 10.90 - 47.27 per cent against *R. solani* and 11.11 - 50 per cent against *R. solanacearum* thus substantiating the suppressive activity of the degraders against soil borne pathogens.

The application of these composts will enhance the plant health and impart disease resistance by their nutrients and biochemical contents. Reading back the results on the nutrient analysis of various composts, it is observed that, high content of available nitrogen, especially in ayurvedic compost might have resulted in enhanced crop health and yield. The available potassium as well as lignin and tannin contents might also have imparted resistance to various diseases. Another factor for the disease resistance may be due to activation of induced systemic resistance in the compost treated plants as reported by Zhang *et al.* (1996); Sang *et al.* (2010) and Yogev *et al.* (2010).

Availability of cow dung is an emerging problem at present. This study revealed that, the cow dung for the aerobic composting of organic wastes can be substituted with a microbial consortium, which will not only reduce the composting period, but also enhance the quality of compost with enrichment of primary, secondary and micronutrients. The application of such composts also provided promotion of plant growth and suppression of soil borne diseases. Hence, the present study is of significant importance in sustainable agriculture.

# *Summary*

# 6. SUMMARY

Lignin-tannin rich agrowastes generated from various sources pose serious threat to the environment, due to lack of sustainable management practices. Decomposition is a natural process where complex organic matter is broken down into simpler compounds by the aid of various microorganisms. The exploitation of metabolic versatility of microorganisms in consortium is advantageous for the rapid decomposition of various organic wastes. Soil borne pathogens are the major limiting factors in agriculture that are often difficult to control with the use of chemical fungicides. Biocontrol with the use of composted organic matter has been proposed as an effective method for the management of many soil borne pathogens. With this view, a study was conducted on the decomposition of various ligno-phenolic agrowastes using microbial degraders and the management of two important soil borne pathogens *viz. Ralstonia solanacearum*, causing bacterial wilt of tomato and *Rhizoctonia solani*, causing leaf blight of amaranth.

- Samples of soils and degraded substrates were collected from 15 sources of 34 locations throughout Kerala and border areas of Tamil Nadu for the isolation of microbial degraders.
- Maximum population of cellulose (562 $x10^{7}cfug^{-1}$) and lignin (684 x $10^{-4}$ $cfug^{-1}$) degraders were recorded in samples of ayurvedic pharmaceutical units whereas, tannin degrader population was highest in the samples of teak plantation (680 x $10^{-4}cfug^{-1}$).
- Microbial population was higher in degraded substrates than in the soil samples. Among the samples, those from ayurvedic units showed maximum population and diversity of cellulose, lignin and tannin degraders.
- Total of 378 microbial degraders which included 125cellulose, 133 lignin and 120 tannin were isolated from various samples from different locations.
- Fungi were the most effective degraders of cellulose, lignin and tannin with largest diffusion zones on selective medium.

- Actinomycetes were the predominant degraders of cellulose whereas fungi dominated the population of both lignin and tannin degraders. Major fungal degraders of cellulose, lignin and tannin belonged to *Aspergillus* spp. and *Penicillium* spp. *Aspergillus* spp. were found to be the effective ones.
- Thirty five degraders selected from *in vitro* studies showed the ability to degrade all the three chemical components on selective medium.
- *In vitro* decomposition of host substrates showed that, **12** degraders of various sources were effective in degrading their host substrates with lowest maturity period. Among these, six degraders of ayurvedic waste and five of coir pith were found to be most effective with composting periods of 40 and 43 days respectively.
- Most of the degraders which were effective on the host substrates under laboratory condition performed well under *in vivo* condition also. Among the degraders, ALF-26, CLF-13, ECF-2, TLF-8, ECA-8 and CaLF-5 were the promising ones.
- The degraders which were effective on their respective host substrates also showed degrading ability of other lignin-tannin rich substrates and the lowest composting period were recorded by TLF-8, CLF-13, CCB-8, CaLF-5 and ATY-1 on various substrates.
- All the selected degraders were found mutually compatible.
- Characterisation of degraders selected for consortium revealed fungal degraders as *Trichoderma harzianum*, *Paecilomyces variotii*, *Penicillium oxalicum*, *P. chrysogenum*, *Talaromyces* sp., *Aspergillus nidulans* and *A. sclerotiorum*. Bacterial degraders included in the consortium were *Arthrobacter* sp. and *Massilia* sp. Three actinomycetes selected belonged to *Streptomyces albus* (2) and *S. niveus* and the yeast was identified as *Candida* sp.
- Two different microbial consortia were developed with and without *Aspergillus* spp. Much variation was not noticed in the composting periods with and without *Aspergillus* spp.

- Among the two, microbial consortium - II consisted of *T. harzianum*, *P. variotii, P. oxalicum, P. chrysogenum, Talaromyces* sp., *Arthrobacter* sp., *Massilia* sp., *S. albus* (2) and *S. niveus* recorded the lowest maturity period of 35 - 57 days for various substrates with minimum period of 35 days in mixed substrate and coir pith under *in vitro* condition.
- Selected consortium was also found effective under *in vivo* conditions with maturity periods 43-65 days, with minimum maturity period in mixed substrate (43 days) and maximum with leaf litters.
- Large scale composting experiment with 20 per cent microbial consortium in comparison with cow dung slurry (20%) and combination of both showed reduction in the composting periods and recorded lowest maturity periods of 30, 35, 40, and 55 days in mixed substrate, coir pith, ayurvedic waste, and leaf litters respectively against 42, 45, 50, and 65 days in cow dung slurry treatment.
- *In vitro* compatibility study of selected degraders with 20 per cent cow dung slurry and supernatant showed that, native microbes of cow dung inhibited certain degraders of consortium and *vice versa*.
- The reisolation of inoculated degraders indicated their viability during the entire period of composting.
- Among the composts treated with microbial consortium, ayurvedic compost was the ideal, with the lowest C:N ratio (10.4:1) and alkaline pH of 8.6.
- Treatment with microbial consortium showed enhancement of NPK in all composts and ayurvedic compost recorded maximum of N and P (4.80 & 0.46 %) whereas, maximum K (1.60 %) was noted in coir pith compost. The composts also recorded presence of various micronutrients.
- Heavy metal contamination in various composts was ruled out with only negligible presence of lead, chromium and nickel.
- Recalcitrant chemicals *viz.* cellulose, lignin and tannin were found reduced in the consortium treated composts and maximum reduction of cellulose

was recorded in ayurvedic compost, lignin in coir pith and tannin in leaf litter composts.

- Composting with talc based formulations @ 1 kg / 100 kg substrate was also equally good as 20 per cent microbial suspension in reducing the composting period. The composting conducted during April – May and October – November recorded variation in composting periods and lowest maturity periods of 32, 34, 34, and 57 days were observed in mixed substrate, coir pith, ayurvedic waste, and leaf litters during October – November.
- The inoculated degraders retained their viability during the entire composting period and were highly thermophilic in nature with temperature tolerance upto 60°C.
- Microbial degraders showed extra cellular enzyme activities in varying proportions.
- All microbial degraders included in the consortium exhibited direct antagonistic potential against major soil borne pathogens. Among the degraders, *T. harzianum* was found to be most effective against all test pathogens.
- In the field trials conducted against leaf blight of amaranth, all compost treatments were effective in reducing disease severity. Among the composts, maximum reduction of severity and the highest yield was recorded for ayurvedic compost.
- Under field conditions all composts showed better management of bacterial wilt and ayurvedic compost was the most effective with respect to disease reduction and yield.

It is concluded that, the microbial consortium developed from the present study led for rapid conversion of agrowastes into composts and these composts provided better management of soil borne diseases, which makes people healthy and farmers wealthy.

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# Appendix

# APPENDIX – I

1. **Dubo's medium** ( Deokar and Sawant, 2004)

| | |
|---|---|
| Di-potassium phosphate | : 0.5 g |
| Sodium nitrate | : 1.0g |
| Magnesium sulphate | : 0.5g |
| Potassium chloride | : 0.5g |
| Ferric sulphate | : Trace |
| Distilled water | : 1000 ml |

2. **Lignin sulphonate medium** (Thimmaiah, 1989)

| | |
|---|---|
| Malt extract | : 15.0 g |
| Tannic acid | : 5.0 g |
| Agar | : 20.0 g |
| Lignin sulphonate | : 1.0 g |
| Distilled water | : 1000 ml |

3. **Tannic ccid medium** (Thormann *et al.*,2002)

| | |
|---|---|
| Tannic acid | : 5.0 g |
| Malt extract agar | : 20.0 g |
| Distilled water | : 1000 ml |

4. **Carboxy methyl cellulose medium (CMC )**

( Guevara and Zambrano, 2006)

| | |
|---|---|
| Sodium nitrate | : 2.0 g |
| Dipotassium phosphate | : 1.0 g |
| Magnesium sulphate | : 0.5 g |
| Potassium chloride | : 0.5 g |
| Carboxy methyl cellulose | : 2.0 g |
| Peptone | : 0.02 g |
| Agar | : 17.0 g |
| Distilled water | : 1000 ml |

5. **Potato dextrose agar medium** (PDA) (Beever and Bollard, 1970)

| | |
|---|---|
| Potato | : 200g |
| Dextrose | : 20.0 g |
| Agar | : 20.0 g |
| Distilled water | : 1000 ml |

6. **Triphenyl tetrazolium chloride (TZC) medium** (Kelman, 1954)

| | |
|---|---|
| Peptone | : 10.0 g |
| Casein hydrolysate | : 1.0 g |
| Glucose | : 5.0 g |
| Agar | : 20.0 g |
| Distilled water | : 1000 ml |

Autoclave 5 ml of 1% Triphenyl Tetrazolium chloride

7. **Czapex –Dox agar medium** (Czapek, 1902)

| | |
|---|---|
| Sodium nitrate | : 2.0 g |
| Dipotassium phosphate | : 1.0 g |
| Magnesium sulphate | : 0.5 g |
| Potassium chloride | : 0.5 g |
| Ferrous sulphate | : 0.01 g |
| Sucrose | : 30.0 g |
| Agar | : 20.0 g |
| Distilled water | : 1000 ml |

8. **Xylan agar medium** (Cai *et al.*,1994)

| | |
|---|---|
| Birch wood xylan | :10.0 g |
| Yeast extract | : 5.0 g |
| Polypeptone | : 5.0 g |
| Dipotassium phosphate | : 1.0 g |
| Magnesium sulphate | : 0.2 g |
| Agar | : 15.0 g |
| Distilled water | : 1000 ml |

9. **Skim milk agar medium** (Singh and Saxena, 2011)

| | |
|---|---|
| Skim milk powder | : 28.0 g |
| Casein hydrolysate | : 5.0 g |
| Yeast extract | : 2.5 g |
| Dextrose | : 1.0 g |
| Agar | : 15.0 g |
| Distilled water | : 1000 ml |

# EFFICACY OF LIGNO-PHENOLIC COMPOST IN THE SUPPRESSION OF SOIL BORNE PLANT PATHOGENS

By

**GLEENA MARY CF**
**(2010-21 -108)**

## ABSTRACT OF THE THESIS

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# ABSTRACT

The study on the "Efficacy of ligno-phenolic compost in the suppression of soil borne plant pathogens" was carried out at College of Horticulture, Vellanikkara during 2011 -2014. The major objectives were to develop a microbial consortium for rapid composting of lignin-tannin rich agrowastes and also to study the effect of these composts on the management of leaf blight of amaranth and bacterial wilt of tomato.

Sample of soils and partially degraded substrates were collected from 15 sources of 34 locations. Among them, ayurvedic wastes yielded maximum population of cellulose and lignin degraders whereas, tannin degraders were more in the samples of teak plantation. A total of 378 microbial degraders were isolated which included 125 cellulose, 133 lignin and 120 tannin degraders. Actinomycetes were the predominant degraders of cellulose and fungi were dominant among both lignin and tannin with majority belonged to *Aspergillus* spp. and *Penicilium* spp. *In vitro* evaluation of degraders on respective selective media showed diffusion zones more than 4cm by 31 cellulose, 39 lignin and 33 tannin degraders. Fungi were the most efficient degraders of cellulose, lignin and tannin. Thirty five degraders found effective on their respective selective media showed ability to degrade all the three chemical components.

*In vitro* decomposition of 33 degraders on respective host substrates recorded lowest maturity periods of 40 and 43 days for the degraders isolated from ayurvedic waste and coir pith. The degraders, ALF-26, CLF-13, CCB-8, ECF-2, ECA-8, TLF-8, CaLF-5 and ATY-1were most effective on their respective host substrates and also on other tested lignin-tannin rich substrates *in vivo*. All the 22 selected degraders were found mutually compatible. Based on the ability to degrade all the three chemical components, early maturity of composting, type and species of organisms and mutual compatibility, 10 degraders *viz. Trichoderma harzianum, Paecilomyces variotii, Penicillium oxalicum, P. chrysogenum, Talaromyces* sp., *Streptomyces albus, S. niveus, Arthrobacter* sp. and *Massilia* sp.were selected for the formulation of microbial consortium.

Screening of selected consortium under *in vitro* and *in vivo* condition showed early maturity of 35 and 43 days in mixed substrate followed by coir pith with 35 and 45 days. In large scale composting of various agrowastes with microbial consortium, cow dung slurry and the combination of both, the minimum maturity period was observed with microbial

consortium as compared to other treatments recording 30, 35, 40 and 55 days for mixed substrate, coir pith, ayurvedic waste and leaf litters respectively. Composting with microbial consortium resulted in drastic reduction of C:N ratio of the composts with 47 -81 per cent reduction from the initial and the lowest in ayurvedic compost (10.4:1) .Composts treated with microbial consortium recorded neutral to alkaline pH and high contents of N, P and K with maximum N and P in ayurvedic and K in coir pith compost. Traces of lead detected in all compost samples were below the permissible limits. Composting with consortium showed reduction in cellulose, lignin and tannin contents with maximum reduction in ayurvedic (49.38 %), coir pith (49.02%) and leaf litter composts (98.95%) respectively. The degraders of the consortium were thermophilic and recorded extra cellular enzyme activities of cellulase, lignin peroxidase, polyphenol oxidase, amylase, catalase, protease and xylanase. The selected degraders showed antagonism against six major soil borne pathogens *viz. Pythium* sp., *Phytophthora* sp., *Rhizoctonia* sp., *Fusarium* sp., *Sclerotium* sp. *and Ralstonia solanacearum* and *T. harzianum* was the most effective one.

In the field evaluation of various composts in the management of bacterial wilt of tomato and leaf blight of amaranth, application of ayurvedic compost was effective in reducing both diseases. In addition, coir pith and leaf litter composts also reduced bacterial wilt incidence and mixture compost in case of leaf blight.