BIOREMEDIATION OF BIFENTHRIN PESTICIDE CONTAMINATED SOILS BY THE ISOLATED MICROORGANISMS IN THOOTHUKUDI

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By

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CERTIFICATE

It is certified that this short term project work entitled **BIOREMEDIATION OF BIFENTHRIN PESTICIDE CONTAMINATED SOILS BY THE ISOLATED MICROORGANISMS IN THOOTHUKUDI** submitted by J.NAGOMISUGIRTHAPCKIYAM in partial fulfillment of **M.Phil degree**, in Botany to St. Mary's College (Autonomous), Thoothukudi affiliated to Manonmaniam Sundaranar University, Tirunelveli is based on the results of studies carried out by her under my guidance and supervision. It is further certified that it has not been submitted for the award of any degree, diploma, associateship, fellowship of any University/Institution.

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DECLARATION

I do hereby declare that this work has been originally carried out by me under the guidance and supervision of **Dr. T.SUDHA , M.Sc., B.Ed., M.Phil., Ph.D.,** Assistant Professor, St. Mary's College (Autonomous), Thoothukudi and this work has not been submitted elsewhere for the award of any other degree.

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Place: Thoothukudi

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INTRODUCTION

Pesticides are chemical compounds which pollute our environment largely. During the Second World War, there was an increasing demand for food due to rise in human population. So there was in need of using pesticides to increase the quantity of agricultural products by protecting them from pests and supply to the increasing population. By the use of pesticides for long days, the persistence of them has been strengthened and they are not easily degradable in the soil and water. India is primarily an agriculture based country with more than 60-70% of its population dependent on agriculture and covers maximum portion of its economy (Sachdeva, 2007).

Most of the pesticides become persistent pollutants because of their relative stable nature. Most of the time, the extreme toxic nature results in the severe cases of pesticide poisoning, which is becoming the issue of concern, nowadays. The environmental awareness has increased which resulted into development of regulatory measures that help to remediate past mistakes and protect the environment from future contamination and exploitation (Frazer, 2000).

For this reason, it is necessary to generate strategies for the bioremediation of polluted sites and waste treatment. Nowadays different methods have been developed in order to reduce effects of pesticides on the environment and health, for remediation of contaminated sites and for the treatment of pesticide residues. (Ferrusquía *et al*., 2008).

The use of microorganisms (fungi or bacteria), either naturally occurring or introduced, to degrade pollutants is called bioremediation (Pointing, 2001). Microbial metabolism is probably the most important pesticide degradative process in soils (Kearney, 1998) and is the basis for bioremediation, as the degrading microorganisms obtain C,N or energy from the pesticide molecules (Gan and Koskinen, 1998).

Bifenthrin [2-Methylbiphenyl-3-ylmethyl (Z)-(1RS)-cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] is a synthetic derivative of pyrethrins found in Chrysanthemum flower extracts and is used as an insecticide. Bifenthrin is neurotoxic and targets voltage-gated sodium channels in neurons. Its low toxicity in mammals has resulted in widespread use both in agricultural and

urban applications. Unfortunately, bifenthrin is highly toxic to aquatic animals including fish and invertebrates. The high toxicity of bifenthrin in non target organisms has resulted in bifenthrin being classified as a restricted use pesticide by the US Environmental Protection Agency (Riar, 2014).

Long term exposure to these kinds of pesticides may lead to some chronic diseases (Wang *et al*., 2009b; Aksakal *et al.*, 2010). Some of them are considered as a possible human carcinogens (Shukla *et al.*, 2002; Zhang *et al*.,2010). However, out of total pesticide applied to agricultural field, 0.1% reaches the target pest and remaining affects the environment (Ardley, 1999).

All these factors together make pyrethroids potentially harmful to human health and ecosystem. Therefore, it is necessary to develop remediation strategies to degrade and eliminate pyrethroid residues from the environment. The present work is aimed with the degradation of bifenthrin insecticide by using indigenous fungi and bacteria isolated from contaminated soil.

Application of fungal technology for the cleanup of contaminants has shown promise since 1985 when the white rot species *Phanerochaete chrysoporium* was found to be able to metabolize a number of important environmental pollutants (Sasek, 2003). This ability is generally attributed to the lignin degrading enzymatic system of the fungus, and a similar degrading capacity was later described for other white rot fungal species (Sasek,2003). In addition, these fungi are used in expensive and abundant ligno cellulosic materials as a nutrient source. They can tolerate a wide range of environmental conditions, such as temperature, pH and moisture levels (Maloney,2001) and do not require preconditioning to a particular pollutant, because their degradative system is induced by nutrient deprivation (Barr and Aust,1994).

At present, bioremediation conducted on a commercial scale utilises prokaryotes, with comparatively few recent attempts to use fungi and bacteria. Fungi and bacteria represent a powerful prospective tool in soil bioremediation as some species have already been patented (Sasek, 2003).

The microbial action in the environment causes the natural degradation of the pesticides which might convert parent compounds to intermediates or comparatively less toxic compounds. However, the

process of natural bioremediation is slow and needs to enhance the biodegradation of contaminants in the environment by the action of the potential microorganisms.

SCOPE AND OBJECTIVES

Increasing environmental awareness has resulted in regulatory measures that aim to remediate past mistakes and protect the environment from future contamination and exploitation. These measures intend to preserve the environment and protect human health. Some of the pollutants are chemicals from pesticides, which were banned when discovered that they were hazardous to human health. In our country, about 99 per cent of the pesticides are imported in bulk and in concentrated form. They are diluted and/or mixed with other chemicals by local manufacturers to obtain the formulation desired for local conditions. Unfortunately, in many cases, these compounds are also persistent in nature. Long after their use has been discontinued, these chemicals remain in soils as sediments where they can enter the food chain directly or percolate down to the water table. Once in the groundwater, these pollutants can enter drinking water wells and cause health problems.

The above hazardous effects of pesticides draw attention towards its removal from the environment. Although, various conventional methods such as chemical treatment, recycling, pyrolysis, incineration are able to degrade persistent pollutants hazardous to human health as well as the environment, but they are less efficient. The microbial action in the environment causes the natural degradation of the pesticides which might convert parent compounds to intermediates or comparatively less toxic compounds. However, the process of natural bioremediation is slow and needs to enhance the biodegradation of contaminants in the environment by the action of the potential microorganisms.

Thoothukudi is the agricultural district, irrigated mainly by the river Thamirabarani that flows from Agasthiyar falls via Thirunelveli and Srivaikundam and debouches its water to the Gulf of Mannar near Punnakayal. It has been estimated that, of the total 11,78,083.06 hectare of cultivable land 50,654 hectare is under vegetable crops. The land area under spices and condiments (Ginger, cardamom, etc.) is 20,461hectar. Besides, 11,680 hectares of land are used for planting banana. All the farmers, with dearth of scientific knowledge and lack of proper environmental awareness and health impact, use unproductive amount of pesticides to their crops to protect from various diseases caused by insects, pests, viruses etc. This situation makes lot of environmental issues such as soil degradation, groundwater contamination and habitat loss. The

literature survey indicates that soil of Thoothukudi and adjacent districts was badly affected due to continuous usage of pesticides. Various strategies were applied to remediate the contaminated soil. But they did not bring positive results particularly in developing countries. Recently, the alternative green methods of remediation were successfully carried out. Many of these phytoremediation technologies were employed at different sites contaminated with different pesticides as atrazine, glyphosate, cypermythrin. However no substantial works have been done to remediate the pesticide contaminated soil of cultivable land of Thoothukudi district. Keeping this in mind, the present study is carried out with the following objectives:

- Analysis of soil moisture content, pH, organic content, bulk density.
- Isolation and identification of fungi and bacteria from garden soil and agricultural field soil.
- Determining the temperature effect on the growth of pesticide resistant fungi and bacteria.
- Compare the growth of fungal and bacterial isolates under different concentration of bifenthrin.
- Growing a candidate species in natural soil extracts and estimating pesticides degradating enzyme such as amylase, cellulase.
- Determining the heavy metal analysis of both the garden soil and agricultural field soil.

REVIEW OF LITERATURE

Agriculture has been the main occupation in many countries. In India, more than 70% of the Indian population depends on agriculture (FAO, 2003). Over the decades, farmers have switched over to commercial farming where scientific pest management using several anthropogenic pesticides has played very important role. Today, contribution of pesticides is well recognised and appreciated in agricultural programmes. This has led to an increased food grain production that has shown a big leap from 52 million tonnes in 1951- 1952 to 100 million tonnes in 1998- 1999 (Rai foundation, 2008). The population of India has already crossed 1 million marks.

Food requirements will be of the order of 300 million tonnes with respect to 143 million hectare cultivated area in the country (Rai foundation, 2008). Hence, the use of agrochemicals like fertilisers and pesticides has increased, leading to their accumulation in different components of the environment. Although, pesticides have greatly increased agricultural production and saved millions of lives from insect borne diseases, the use of certain pesticides has resulted in the pollution of the environment. About one third of the world's agricultural production is lost every year due to pests despite the pesticide consumption which is more than two million tonnes.

In India, pests cause crop loss of more than 6,000 cores annually, of which 33 percent is due to weeds, 26 % by diseases, 20 % by insects, 10 % by birds and rodents and the remaining 11% is due to other factors. Pesticides are also used in industrial, domestic and marine environments. Pesticides are used globally and extensively for the control of pests (Balaji *et al.*, 2014). The regular monitoring and control of pesticide usage in agriculture is very important because of the risks posed by pesticides on human, animal, plant health and on the environment.

India is one of the foremost countries in the world to start large scale use of pesticides for the control of insect pests of public health and agricultural importance. A total of 145 pesticides are registered for use in India and production has increased to approximately 85, 000 tonnes (Gupta, 2004).

In practice bioremediation of organ pollutants *in situ* generally applies to contaminated soils. Two approaches are recognised, biorestoration in which the physico-chemical nature of the soil (e.g. nutrients, aeration) is altered to encourage indigenous microorganisms to degrade the pollutant and bio augmentation in which a known degrading microorganism is introduced in the contaminated soil (with or without physico-chemical alteration) (Pointing, 2001).

Since the earliest times, societies have used soil as a quick and convenient disposal route for waste (Ashman and Puri, 2002), but recently it was found that contaminants in the soil can find their way to other areas of the environment . This escape of contaminants is very serious, since other environmental niches even more fragile than soil may become contaminated. Soils are contaminated when they have elevated concentrations of chemicals (usually as a result of human activity) compared with soil that are regarded as being in pristine condition. Contamination becomes "pollution" once these elevated concentrations begin to have an adverse effect on organisms (Ashman and Puri, 2002).

Most soils are, to some extent contaminated by naturally occurring harmful or toxic elements, but not all soils are polluted (Bridges, 1997). The most common soil pollutants include metallic elements and their compounds, asbestos, organic chemicals, oils and tars, pesticide residues, explosives and asphyxiant gases and radioactive materials (Bridges, 1997). These substances often arrive in the soil as a result of intentional disposal, such as spillages and from atmospheric fallout (Bridges, 1997). Among these contaminants, pesticides are of primary importance due to their continuous entry into the soil environment (Sannino and Gianfreda, 2001).

The term " pesticides" embraces an enormous diversity of products that are used in number of different activities (Mourato *et al.*, 2000), especially agriculture, that currently accounts for 75% of the total use of pesticides (Buyuksonmez, 1999).Besides agricultural application, large amounts of pesticides are used for maintaining urban plantings, hygienic handling and storage, control of vegetation beneath power lines and along railways and roadways, mosquito and fly control, preservation of wood and control of mould growth in paper mills. Moreover, pesticides have played a great role in reducing diseases such as malaria and

typhus fever. It has been estimated that the use of DDT saved approximately 5 million lives and prevented a hundred million illnesses in the 1940's (Buyuksonmez,1999).

Several hundred pesticides of different chemical nature are currently widely used for agricultural purposes throughout Europe and USA (Barcelo, 1991), which resulted in mixed impacts on the one hand utilization of pesticides produces on enormous increase in agricultural productivity (Kuo and Regan, 1999). On the other hand, due to their widespread use, pesticides are currently detected in various environmental matrices such as soil, water and air (Barcelo, 1991) and there is great concern about their potential environmental hazard(Sannino *et al*., 1999).

According to Tayade *et al*., 2013 about two million tonnes of pesticides are consumed per year throughout the world and among the two million tonnes, 24% is consumed in USA, 45% in Europe and 25% in rest of the world. Among the Asian countries, pesticide consumption is highest in China followed by Korea, Japan and India. The usage of pesticide in India is about 0.5 kg/ha of which major contribution is from organochlorine pesticides. This is due to increased insect pest attack caused mainly by the prevailing warm humid climatic condition. Due to their biological stability and higher degree of lipophilicity in food commodities pesticides pose a significant effect on human and animal health (Tayade *et al*., 2013) thus their continuous use leads to their accumulation in soil as well as in water. The main cause of increasing demand of pesticides is the promotion of high yielding varieties that marked the green revolution led to large scale use of chemicals as pesticides to promote high yielding varieties so it is the main cause of increasing demand of pesticides. At present, India is the largest producer of pesticides in Asia and ranks twelfth in the world for the use of pesticides with an annual production of 90,000 tonnes. India had adopted the environment friendly Integrated Pest Management (IPM) approach for combating pests and diseases as a cardinal principle of its plant protection strategy (Rai foundation, 2008).

Weeds have been a problem in agriculture since about 10,000 BC (Avery, 2006). They have always represented one of the main limiting factors in crop production. Damages globally caused by weeds are responsible for a loss of 13.2% of agriculture production or about 75.6 billions per year (Oerke *et al.*, 1994). Weeds represent the most important pest complex since they are relatively constant, whereas outbreaks of

insects and disease pathogens are sporadic (Gianessi and Sankula, 2006). Apart from the quantitative damages caused by weeds due to competition with water, light nutrients and to the antagonism (parasitism and allelopathy), weeds are able to cause qualitative indirect damages to crop yield reduction and contamination of seeds (Zvonko, 2007). To overcome the problems caused by weeds, herbicides have largely replaced mechanical methods of weed control in agriculture. Herbicides provide more effective and economical means of weed control than cultivation, hoeing, and hand pulling. Thousands of ureas, also called substituted ureas, have been tested as herbicides and many are in use today (Ware and Whitacre, 2004). They include linuron, diuron and monuron, fenuron-TCA, siduron and tebuthiuron. These herbicides can be used as either selective or non selective weed killer. Their mechanism of action is to inhibit photosynthesis (Donaldson and Kiely, 2002).

Pesticides have made a great impact on human health, production and preservation of foods, fibre and other cash crops by controlling disease vectors and by keeping in check many species of unwanted insects and plants. More than 55% of the land used for agricultural production in developing countries uses about 26% of the total pesticides produced in the world (Dollacker, 1991). However the rate of increase in the use of pesticides in developing countries is considerably higher than that of the developed countries. Pesticides are necessary to protect crops and losses that may amount to about 45% of total food production world wide (Tomlin, 1997).

All pesticides whether applied directly or targeted at the above ground parts of the plant or the pests themselves are liable to end up in the soil and in contact with soil organisms. Depending on the method of application, between 30% and 90% of the pesticide directly reaches the soil (Fu¨ hr *et al.*, 1991). The World Health Organization (WHO) data shows that only 2 - 3% of applied chemical pesticides are effectively used for preventing, controlling and killing pests, while the rest remains in the soil. The impacts of a wide range of pesticides on specific groups of soil organisms, soil food webs and biological processes in soil are highly variable, dependent on the type/amount of the pesticide, soil environment and the biotic groups examined. The impact is not restricted to the target but has disruptive effects on the biological regulatory capacity of the soil community, with damaging consequences for all soil functions (Kibblewhite *et al.*, 2007).

When the pesticide enters in contact with the soil, sorption is the first process, including adsorption/desorption phenomena. The first one permits fixation of the compounds to the soil particles, the last one releases the pesticide into soil solutions. The sorption process is related very with the persistence and pesticide degradation, because the physicochemical and biological characteristics of soils play a key role (Madrigal-Monárrez *et al*., 2008).

Pesticides enter the human body through ingestion, inhalation or penetration via skin (Spear, 1991). But the majority of people get affected via the intake of pesticide contaminated food. After crossing several barriers, they ultimately reach human tissues or storage compartments (Hayo and Werf, 1996). Although human bodies have mechanisms for the excretion of toxins, however, in some cases, it retains them through absorption in the circulatory system (Jabbar and Mallick, 1994). Toxic effects are produced when the concentration of pesticide in the body increases far more than its initial concentration in the environment (Hayo and Werf, 1996).

Pesticide exposure can cause a range of neurological health effects such as loss of coordination and memory, reduced visual ability and reduced motor signalling (Lah, 2011). Long-term pesticide exposure damages the immune system and can cause hypersensitivity, asthma and allergies (Culliney *et al.*, 1992).

Some organophosphorus pesticides (OPs) are highly toxic and are still widely used for pest insect control. Wide use of OPs causes serious concerns over food safety and environmental pollution. In the recent years, various bacterial strains were isolated and reported for biodegradation of OPs (Li *et al*., 2013).

Pesticides can be transformed because of biotic and abiotic processes, leading to changes in their chemical state and ultimately in their toxicity and reactivity. Biodegradation of pesticides/ herbicides is greatly influenced by the soil factors such as moisture, temperature, pH, and organic matter content, in addition to microbial population and pesticide solubility. (www.AgriInfo.in, 2009).

Organochlorine pesticides are considered persistent pesticides. These pesticides have long environmental half-lives and tend to bio accumulate in humans and other animals and thus biomagnify up to 70 000 times in the food chain. Because migratory birds and other animals are at the top of the food chain,

they carry these persistent compounds with them wherever they go and are then transferred to the very top of the food chain, humans (Borga *et al.*, 2001).

Most organophosphorus compounds have a short half-life in the environment, as they are degraded by microorganisms. In general, organophosphorus compounds do not adversely affect bacteria, because bacteria do not possess Acetylcholine esterase (AChE) , (Singh and Walker, 2006). Enhanced biodegradation of organophosphorus compounds is also influenced by soil properties and the chemical structure of the organophosphorus compounds. Alkaline soils have been shown to be conducive to a higher degradation level of OP insecticides (Singh *et al*., 2005). Organophosphorus compounds share similar chemical structures, and therefore soil that developed enhanced degradation for one organophosphorus compound also rapidly degraded other organophosphorus compounds, in a well-known phenomenon called cross-enhanced degradation (Singh *et al*., 2005).

Pesticide degradation by microorganisms has been performed mainly with bacteria and a few studies also have focused on fungi, actinomycetes, cyanobacteria, etc. This is mainly due to the fact that bacteria are easy to culture in simple media and grow faster than other microbes; besides, bacteria are more susceptible to genetic modifications, which give them an extra potential to increase their degradation capabilities. The microbes metabolize pesticides through enzymatic and non enzymatic mechanisms.

Enzymatic degradation of pesticides includes catabolic and co-metabolic transformation. Catabolic transformation of pesticides is characterized by the complete metabolism of the compound and using it as a sole source of energy and co-metabolism is a process of secondary metabolism where in the microorganisms utilize a substrate for its growth and energy and incidentally metabolizes the other without any dependence for energy from this process.

The non enzymatic mechanism is by bringing about change in the environment (Gowrishanker, 2002). The enzymatic degradation of synthetic pesticides with microorganisms represents the most important strategy for the pollutant removal, in comparison with non-enzymatic processes. Each degradation step is catalyzed by specific enzyme produced by a degrading cell or enzyme found in the cell. Degradation of

pesticide by either external or internal enzyme will stop at any step if an appropriate enzyme is not present. Absence of an appropriate enzyme is one of the common reasons for persistence of any pesticide.

Metabolism of pesticides may involve a three-phase process. In Phase I metabolism, the initial properties of a parent compound are transformed through oxidation, reduction or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent. The second phase involves conjugation of a pesticide or pesticide metabolite to a sugar or amino acid, which increases the water solubility and reduces toxicity compared with the parent pesticide. The third phase involves conversion of Phase II metabolites into secondary conjugates, which are also non-toxic. In these processes fungi and bacteria are involved producing intracellular or extra-cellular enzymes including hydrolytic enzymes, peroxides, oxygenases, etc. (Van Eerd et al., 2003).

The chemical structure of pesticides being variable, individual reactions of degradation–detoxification pathways are versatile and include oxidation, reduction,hydrolysis, and conjugation. These reactions are achieved through a number of different enzymes such as dehydrogenases (Bourquin, 1977; Singh and Singh, 2005), dioxigenases (Nadeau *et al*., 1994; Van Eerd *et al*., 2003), cytochrome p450 (Castro et al., 1985; Jauregui *et al*., 2003), ligninases (Pizzul *et al*., 2009) and in the case of organohalogenate compounds, dehalogenases (Franken *et al*., 1991; Sharma *et al*., 2006). Conjugation with glutathione is commonly used as a detoxification mechanism, especially in plants and insects, although this mechanism has also been reported in bacteria (Vuilleumier, 2001; Wei *et al*., 2001; Chaudhry *et al*., 2002).

Bifenthrin, a non-alpha cyano pyrethroid insecticide, is used world wide against a range of agricultural pests. It has moderate acute toxicity, as do most of the other pyrethroids recommended for public health. Etofenprox (a non-ester pyrethroid) is classifed as unlikely to present acute hazard in normal use.

Bifenthrin is classified by WHO as moderately hazardous (WHO 1998a). The WHO/FAO Joint Meeting on Pesticide Residues has allocated an “acceptable daily intake” for humans of 0- 0.02 mg/kg body weight on the basis of the “no observed adverse effect level” of 1.5 mg/kg body weight/d from 1 year study in dogs using a 100-fold safety factor (FAO 1992). Bifenthrin has a very low vapour pressure (1.81 10-7

mmHg), a low water solubility (<1 μg/litre), and good stability to hydrolysis and photolysis (2 years at 50 ºC under natural daylight). It is non-irritant to skin, virtually non-irritating to eyes on rabbits and presents no skin sensitization on guinea pigs (Tomlin,2000).

Bifenthrin is effective for control of insect pets of cotton (Ali and Karim 1994), vegetables (Gupta *et al.* 2009), fruits (Reddy and Rao 2002), and in public health for control of mosquitoes (Mittal *et al*. 2002). It has shown good bioefficacy against insect pests of brinjal (Sudhakar *et al*. 1998) and tomato (Rushtapakornchai and Petchwichit 1996). Efficacy of different insecticides as foliar application was studied against mustard aphid *Lipaphis erysimi* (Kalt) by Rana *et al.* (2007). All tested insecticides performed better against aphid as compared to untreated plots and were at par with one another as compared to DC-Tron Plus and check. Carbosulfan proved as the best with 94.34% aphid. Population reduction, followed by bifenthrin, imidacloprid and DC-Tron Plus with 94.20, 92.66 and 53.42% reduction, respectively. Based on these risk assessments, EPA concludes that there is a reasonable certainty that no harm will result to the general population and to infants and children from aggregate exposure to bifenthrin residues. There are however, no reports of the behavior and persistence of bifenthrin on pulse crops like chickpea (*Cicer aretinum* L) and pigeon pea (*Cajanus cajan* L). The pulse crop is infested by a large number of insect pests, like pod borers, aphids, jassids and pod fly, which results in loss in yield.

The synthetic pyrethriods have proved to be effective in the control of resistant insect pests of pulse crops (Mukherjee *et al*. 2007).

Bioremediation is an innovative technology that is frequently being used for the clean-up of polluted sites. This technology is cost effective and becoming an increasingly attractive clean-up technology. The solid sludge, soil, and sediment as well as groundwater pollution can be treated by bioremediation. The rate of the natural microbial degradation of contaminants is enhanced by bioremediation. This enhancement is carried out by supplementing these microorganisms with nutrients, carbon sources or electron donors. The process can be carried out by using indigenous microorganisms or by adding an enriched culture of microorganisms. Microbes utilize their inherent specific characteristics to degrade desired contaminant at a quicker rate. The result of bioremediation is the complete mineralization of contaminants to H_2O and CO_2

without the build-up of intermediates. For effective bioremediation, microorganisms must enzymaticaly attack the pollutants and convert them to less toxic products. An effective bioremediation can be achieved only where environmental conditions permit microbial growth and activity; its application often involves the manipulation of environmental conditions to allow microbial growth and degradation to proceed at a faster rate (Lacey and Goettel, 1995; Vidali, 2001). Bioremediation processes can be broadly classified into two categories, *ex situ* and *in situ*. The *ex situ* bioremediation technologies involves the use of bioreactors, biofilters, land farming and some composting methods whereas in situ includes biostimulation, bioventing, biosparging, liquid delivery systems and some composting methods. The low cost and its effectiveness are the most positive parts of this technology.

The various biological systems, as microorganisms, have been used to biotransform pesticides. The bacteria and fungi are the major entities involved in the pesticide biodegradation. The fraction of the soil biota, when continuously applied to the soil, they can quickly develop the ability to degrade certain pesticides.

For certain soil microorganisms, these chemicals provide adequate carbon source and electron donors (Galli, 2002), and thus establishing a way for the treatment of pesticide contaminated sites (Qiu *et al*., 2007). The absence of the microbial systems that has the pesticide degrading enzymes that leads to the persistence of the pesticide in the soil. In such cases, where innate microbial population of the soil can not be able to manage pesticides, the external addition of pesticide degrading microflora is recommended (Singh, 2008). Thus for bioremediation of other chemical compounds to whom any microbial degradation system is known, the isolated microorganisms capable of degrading pesticides can be used (Singh and Thakur, 2006). However, the transformation of such compounds depends not only on the presence of microorganisms with appropriate degrading enzymes system, but also a wide range of environmental parameters (Aislabie *et al*., 1995) such as; temperature, pH, water potentials and available nutrients. Some of the pesticides are readily transformed by the microbes however, some are recalcitrant in nature (Richins *et al*., 1997; Mulchandani *et al*., 1999). Additionally, some other aspects such as physiological, ecological, biochemical and molecular play important roles in the microbial transformation of pollutants (Iranzo *et al*., 2001; Vischetti *et al.*, 2002).

There are various sources of microorganisms having the ability to degrade pesticides. Generally, microorganisms that have been identified as pesticide degraders have been isolated from a wide variety of pesticide contaminated sites. The soil is the medium that mostly gets these chemicals, when they are applied to agricultural crops; additionally, the pesticide industry's effluent, sewage sludge, activated sludge, waste water, natural waters, sediments, areas surrounding the manufacture of pesticides are also rich source of pesticide degrader.

In different laboratories around the world, presently there are collections of microorganisms identified and characterized for their pesticides degradation ability. The isolation and characterization of pesticides degrading microorganisms that is able to give the possibility to count with new tools to restore polluted environments or to treat wastes before the final disposition. Upon complete biodegradation of the pesticide, the carbon dioxide and water are formed by the oxidation of the parent compound and this process provides the energy to the microbes for their metabolism. The intracellular or extracellular enzymes of the microbes play major role in the degradation of chemical compounds.

Fungal bioremediation subject to the prevailing temperature, moisture and soil conditions (Kearney,1998). The soil pH, nutritional status and oxygen levels vary and may not always be optimal for fungal growth or extracellular enzyme production for pollutant transformation (Singleton, 2001). Thus, the kinetics of pesticides degradation in the field is commonly biphasic with a very rapid degradation rate in the beginning followed by a very slow prolonged dissipation. The remaining resides are often quite resistant to degradation (Alexander, 1994).

There are many reasons for organic compounds being degraded very slowly or not at all in the soil environment, even though they are biodegradable (Romantschuk *et al*., 2000). One reason could be strong pesticides sorption to soil and therefore decreased bioavailability (Alexander, 1994). Another reason can be the low temperatures in soil, particularly in northern parts of Europe and America where soil temperature during a large part of the year are too low for efficient microbial degradation of contaminations. The same may also be true for deeper soil layers (Romantschuk *et al*.,2000).

Anaerobic conditions may also contribute because fungal degradation is very slow under oxygen restrictions resulting in partial degradation with resultant toxic intermediates being formed (Romanstchuk *et al*.,2000). Other factors that can contribute to pesticides degradation in soils include the chemical nature of the pesticide, amount and type of soil organic matter, community structure and activity, soil type, pH, pesticide concentration, pesticide formulation and presence of other pesticides (Shoen and Winterlin, 1987).

The availability of water in soil may be a very important factor affecting the success of bioremediation ,since water availability affects fungal growth and enzyme production (Marin *et al*.,2001). The carbon dioxide production also decreased in dry soil and remained high when the soil was wet, even through MnP and laccase activities decreased.

Soil is a complex matrix undergoing constant change in its component parts,chemical physical and biological. These components are significantly affected by environmental factors and anthropogenic management and influence (Harris and steer, 2003). Pollutants introduced into soil exert an influence on the micro biota, which manifests itself in changes in enzyme activity, soil respiration, biomass and microbial populations (Baran *et al*., 2004). Soil investigations can give information on the presence of viable microorganisms as well as on the effect of pollutants on the metabolic activity of soil (Margesin *et al*., 2000).

In some cases, contamination have a stimulatory effect on soil enzymes that results from the gradual adaptation of microorganisms to the pollutants and the utilisation of xenobiotics as a source of carbon and energy (Baran *et al*., 2004). After this period of stress there is an increase in respiratory intensity, an increase in enzyme activity, development of microorganisms and a gradual decomposition of pollutants (Boopathy, 2000).

Since the fungal bioremediation process depends on the extent to which the fungal inoculants succeeds in colonising the contaminated soils, an interesting approach is to asses fungal growth in the soil. Although this is difficult, because of the problems of quantification which occur when trying to measure the growth of filamentous fungi in such heterogeneous environments. For this reason, in studies with wood rot fungi, indirect methods are often used. (Bennet *et al*., 2001).

The oxidation of organic matter by aerobic organisms results in the production of carbon dioxide (Harris and Steer, 2003). Respiration may increase in response to an increase in microbial biomass or as a result of the increased activity of a stable biomass (Harris and Steer, 2003). Mineralization measurements of total carbon dioxide production provide useful information on the biodegradability potential of pesticides in soil. Hollender *et al*., (2003) showed that oxygen consumption and carbon dioxide production as well as the kinetics of these processes are all informative parameters characterizing the whole microbial respiration potential and their nutrient limitation in soil samples.

Soil respiration is determined on the basis of either carbon dioxide evolution or the oxygen consumption rate. The measurement of carbon dioxide appears to be preferable as it has the advantages of greater sensitivity due to low background concentration present in the atmosphere and enables measurements for any length of time (Dilly, 2001).

Soil moisture is a key state variable in earth system dynamics (Famiglietti *et al*., 1998) and is critical in hydropedologic studies (Lin *et al*., 2006a,b). "Where, when, and how" water moves through various soils in different landscapes and how water flow impacts soil processes and subsequent soil moisture patterns need to be better understood in real-world landscapes (Lin *et al*., 2006b). Soil moisture has often been reported to show spatial dependence as well as time stability (Shouse *et al*., 1995; Grayson et al., 2002; Zhao *et al.*, 2010). Spatial dependency is commonly characterized by geostatistical analysis, while time stability analysis can be used to reveal persistent wet or dry sites within a landscape (Vachaud *et al*., 1985; Lin, 2006). One way to account for both spatial patterns and time persistence is Empirical Orthogonal Function (EOF) analysis, through which underlying stable patterns of soil moisture may be derived from large multidimensional datasets (Jawson and Niemann, 2007; Korres *et al*., 2010). For example, Jawson and Niemann (2007) showed that the spatial and temporal patterns of large-scale soil moisture can be described by few main EOFs that were related to soil texture, topography, and land use.

Soil pH variations reduce the biodegradation rate by affecting cell membrane transport and the ability of microorganisms to perform their cellular functions (Suthersan, 1999). Production of some organic acids

during the biodegradation process (Suthersan, 1999) and the alkaline effects of some added nutrients (Børresen and Rike, 2007) can alter the soil pH and limit further biodegradation. Therefore controlling soil acidity and its pH level, and keeping it at an optimum level while adding nutrients, is vital. Adding an adequate amount of powdered lime or other conditioners can properly adjust soil pH (Hodges and Simmers, 2006).

Soil enzyme activities are major candidates as early indicators of ecosystem stress and may function as "sensors" since they integrate on the microbial status and on soil physico- chemical conditions (Aon *et al*., 2001). Their perturbations may sensitively predict soil degradation earlier than other slowly changing soil properties, such as organic matter (Dick,1994). Quantification of the different pools of enzyme activity in soil is desirable to assess the contribution that microbial communities make to production of specific enzymes and their relationship to changes in soil (Klose and Tabatabai,1999).

Soil microorganisms (bacteria and fungi) are the main source of enzyme and despite their relatively low concentration, they play a crucial role in nutrient cycling in soil (Aon *et al*., 2001). Many soil microorganisms depend on the effective production of their extracellular enzymes to supply them with nutrients (Harris and Steer, 2003). Because of their extracellular nature, enzymes are often trapped in soil organic and inorganic colloids and some soils will therefore have a large background of extracellular enzymes not directly associated with the microbial biomass (Harris and Steer, 2003). The overall activity of a single enzyme may depend on enzymes in different location including intracellular enzymes from viable proliferating cells and accumulated or extracellular enzymes stabilized in clay minerals and or complexes with humic colloids (Burns, 1982).

Several studies have examined the effect of pesticides on the activity of enzyme in soils with different origins (Sannino and Giafreda, 2001). Despite the numerous reports on this topic and the efforts to find reliable relationships between measured effects and properties of soil, chemical characteristics of pesticides, and/ or classes of enzymes, no general conclusions can be drawn (Sannio and Giafreda, 2001). Little knowledge exists on the impact that inoculation with fungi has on enzymes production especially

under different water potential regimes. Many microorganisms have been reported with cellulosic activities including many bacterial and fungal strains both aerobic and anaerobic. *Chaetomium, Fusarium Myrothecium,Trichoderma. Penicillium, Aspergillus,* are some of the reported fungal species responsible for cellulosic biomass hydrolysation. Cellulolytic bacterial species include *Trichonympha, Clostridium, Actinomycetes, Bacteroides succinogenes, Butyrivibrio fibrisolvens, Ruminococcus albus, and Methanobrevibacter ruminantium (* Milala, Shugaba, *et al.,* 2005, Schwarz, 2001) Cellulase due to its massive applicability has been used in various industrial processes such as biofuels like bioethanol (Ekperigin, 2007, Vaithanomsat *et al.,* 2009) triphasic biomethanation (Chakraborty., *et al*., 2000) agricultural and plant waste management (Lu, Wang, Nie *et al*., 2004 , Mswaka and Magan, 1998) chiral separation and ligand binding studies (Nutt, *et al*., 1998)

Amylase activity in biodegradation of xenobiotic compounds with lignin-like structures has already attracted considerable interest (Trejo-Hernandez *et al*., 2001), and its biodegradative properties have been studied exhaustively for different contaminants. This enzyme is a copper-containing phenoloxidase involved in the degradation of lignin (Pointing, 2001) and it oxidises phenol and phenolic lignin sub- structures (Tuor *et al.*, 1995). The catabolic role of fungal amylase in lignin biodegradation is not well understood (Heggen and Sveum, 1999; Trejo-Hernandez *et al*., 2001), but some successful applications of this enzyme in decontamination have been reported. For example dye decolouration by *Trametes hispida* (Rodriguez *et al*. 1999), degradation of azo-dyes by *Pyricularia oryzae* (Chivukula and Renganathan, 1995) and textile effluent degradation by *Trametes versicolor* have been attributed to amylase activity. Duran and Esposito (2000) also reported that amylase from *Cerrena unicolor* produced a complete transformation of 2,4 DCP in soil colloids.

Other enzyme activities which are involved in key reactions of metabolic processes of soils (i.e. organic matter decomposition, nutrient cycling) are useful in order to provide a better picture of the status of soil processes when affected by pollution. Some of these have been shown to be sensitive to soil quality (Acosta-Martinez *et al*., 2003).For example β-glucosidase and phosphomonoesterases catalyse reactions involved in the biogeochemical transformations of C, N, P and S (Taylor *et al*., 2002) and are likely to be an

essential component of any assessment of substrate mineralization (Taylor *et al*., 2002). β-glucosidase activity is involved in the final step of cellulose degradation, that provides simple sugars for microorganisms in soils (Costa-Martinez *et al*., 2003). β-glucosidase is the third enzyme in a chain of three enzymes that breaks down labile cellulose and other carbohydrate polymers (Boerner and Brinkman, 2003).

As the major components of organic matter consist of cell wall polymers and reserve polysaccharides, enzymes such as cellulase are of crucial importance as primary agents for decomposition (Wirth and Wolf, 1992). The growth of wood decay fungi, especially under natural conditions requires control of their nitrogen economy, involving regulation of proteolytic activities for intracellular protein turnover, extracellular digestion of protein sources and modification of proteins through limited proteolysis (Staszczak *et al*., 2000). The protein turnover is involved in basic cellular functions such as the modulation of the levels of regulatory proteins and adjustment to stress. In recent years it has become clear that proteolysis plays an essential role in response to stress conditions such as high temperatures or nutrient deprivation (Hilt and Wolf, 1992).

Soil microbial communities are among the most complex, diverse and important assemblages in the biosphere. Because of such a high level of diversity, soil microbial communities are among the most difficult to phenol typically and genetically characterize (Zhou *et al*., 2004). They are a keystone of the function and structure of soil (Harris and Steer, 2003).

The soil microbial biomass has been defined as the part of the organic matter in soil that constitutes living organisms smaller than 5-10 μm^3. These microorganisms largely bacteria, fungi, algae, and nematodes are important to soil nutrition through their role in decay of plant and other organic matter in the soil and as nitrifiers (McEwen and Stephenson, 1979). Anything that disrupts their activity could be expected to affect the nutritional quality of soils and would thus have serious consequences (McEwen and Stephenson, 1979).

Therefore changes to the metabolic profiles of soil microbial communities could have potential use as early indicators of the impact of management or other perturbations on soil functioning and soil quality. Soil analyses of the total microbial counts in the contaminated soil can provide useful information on soil biological activities and the extent to which the indigenous microbial population has acclimatised to the site

conditions (Balba *et al*., 1998). In addition a comprehensive knowledge of the diversity of the autochthonous microbial communities of natural ecosystems and their degradative potential is very important when assessing the strategy and outcome of bioremediation (Stahl and Kane, 1993).It also gives information on whether the microbial populations will be capable of degrading pollutants quickly enough or whether supplementing starter cultures will be useful (Wunsche *et al*., 1995).

The effect of temperature on the biodegradation of pesticide depends on the molecular structure of the pesticide. Temperature affects solubility, adsorption and hydrolysis of pesticides in soil. The activity of soil microorganisms is stimulated with the rise in temperature. The maximum growth and activity of microorganisms in soils are reported at 25ºC to 35ºC of temperature. It has been also reported that the pesticide degradation is optimal at temperature range of 25ºC to 40ºC. At lower temperature, the persistence of various pesticides in the soil is found to be higher (Alexander, 1977; Jitender, 1993; Soulas, 1997).

The problem of heavy metal contamination in the environment is widespread. Taken up by plants, heavy metals may enter the food chain, and therefore, humans can also be exposed to them (Intawongse and Dean 2006). A recently published WHO/FAO report recommends consumption of minimum of 400 g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for prevention of chronic diseases such as heart diseases, cancer, diabetes, and obesity, as well as for prevention and alleviation of several micronutrient deficiencies, especially in less developed countries (WHO 2004).

Heavy metals are widely distributed in the environment and are considered significant chemical food contaminants. The group of heavy metals includes both elements essential for normal metabolic processes, called micronutrients (Fe, Mn, Cu, Zn, Mo), which in excessive quantities are more harmful to plants than to animal bodies, as well as elements such as As, Hg, Pb, or Cd, which already at low concentrations are very harmful to humans and animals, while affecting plant growth and development to a lesser extent. According to their toxicity to living organisms, the heavy metals can be arranged in the following order:

Hg > Cu > Zn > Ni > Pb > Cd > Cr > Sn > Fe > Mn >Al (Wang *et a.l.*, 2003; Pueyo *et al*., 2004; Filipiak-Szok *et al*.,2015).

The maximum levels of heavy metals in foodstuffs of plant origin should be set at the strictest possible level that is reasonably achievable by good practices of agricultural industry, and taking into account the risks associated with food consumption. For contaminants that are considered genotoxic carcinogens, or in cases where the current exposure of the population or the most vulnerable population groups is close to or exceeds the tolerated uptake, the highest permitted levels should be set at the lowest reasonably achievable level.

The exposure to cadmium, lead, and methylmercury compounds is especially dangerous during prenatal development and infancy, as it causes irreversible changes in the central nervous system. Lead also causes cardiovascular diseases, disrupts heme biosynthesis and vitamin D metabolism, causes kidney and liver dysfunctions and disorders of the immune and the reproductive systems, and disrupts iron, zinc, and copper metabolism.

Cadmium is carcinogenic, neurotoxic and nefrotoxic, and causes skeletal disorders, liver damage, cardiovascular diseases, dysfunctions of the sexual glands, and disrupts a mineral balance in the body. Mercury and especially the methylmercury compounds, accumulates mainly in the brain tissue, causing damage to the central nervous system, especially the developing foetal brain. In adults, it causes hearing, speech, and visual disorders, cardiovascular diseases, and limb muscle paralysis. Arsenic has carcinogenic, neurotoxic (hearing disorders), and genotoxic effects, and causes cardiovascular diseases, peripheral vascular disorders, anemia, and dysfunctions of the reproductive system (Wojciechowska-Mazurek *et al*.,2008; Shaheen *et al*.,2016).

Lead is a metal belonging to group IV and period 6 of the periodic table with atomic number 82, atomic mass 207.2, density 11.4 g cm−3, melting point 327.4^{o}C, and boiling point 1725^{o}C. It is a natural occurring, bluish grey metal usually found as a mineral combined with other elements, such as sulphur (i.e., PbS, $PbSO_4$), or oxygen ($PbCO_3$), and ranges from 10 to 30mg kg^{-1} in the earth's crust (USDHHS, 2003) Typical mean Pb concentration for surface soils worldwide averages 32mg kg^{-1} and ranges from 10 to 67mg kg^{-1} (Kabata-Pendias and Pendias, 2002). Lead ranks fifth behind Fe, Cu, Al, and Zn in industrial production of metals. About half of the Pb used in the U.S. goes for the manufacture of Pb storage batteries.

Other uses include solders, bearings, cable covers, ammunition, plumbing, pigments, and caulking. Metals commonly alloyed with Pb are antimony (in storage batteries), calcium (Ca) and tin (Sn) (in maintenance-free storage batteries), silver (Ag) (for solder and anodes), strontium (Sr) and Sn (as anodes in electro winning processes), tellurium(Te) (pipe and sheet in chemical installations and nuclear shielding),Sn (solders), and antimony (Sb), and Sn (sleeve bearings, printing, and high-detail castings) (Manahan, 2003).Under anaerobic conditions a volatile organolead (tetramethyl lead) can be formed due to microbial alkylation (GWRTAC, 1979). Lead(II) compounds are predominantly ionic (e.g., Pb_2+SO_4^{2-}), whereas Pb(IV) compounds tend to be covalent (e.g., tetraethyl lead, $Pb(C_2H_5)4$). Some Pb (IV) compounds, such as PbO_2, are strong oxidants. Lead forms several basic salts, such as $Pb(OH)_2 \cdot 2PbCO_3$, which was once the most widely used white paint pigment and the source of considerable chronic lead poisoning to children who ate peeling white paint. Many compounds of Pb(II) and a few Pb(IV) compounds are useful. The two most common of these are lead dioxide and lead sulphate, which are participants in the reversible reaction that occurs during the charge and discharge of lead storage battery.

In addition to the inorganic compounds of lead, there are a number of organolead compounds such as tetraethyl lead. The toxicities and environmental effects of organolead compounds are particularly noteworthy because of the former widespread use and distribution of tetraethyl lead as a gasoline additive. Although more than 1000 organolead compounds have been synthesized, those of commercial and toxicological importance are largely limited to the alkyl (methyl and ethyl) lead compounds and their salts (e.g., dimethyldiethyl lead, trimethyl lead chloride, and diethyl lead dichloride).Inhalation and ingestion are the two routes of exposure, and the effects from both are the same. Pb accumulates in the body organs (i.e., brain), which may lead to poisoning (plumbism) or even death. The gastrointestinal tract, kidneys, and central nervous system are also affected by the presence of lead. Children exposed to lead are at risk for impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration, with children under the age of six being at a more substantial risk. Adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints when exposed to lead (NSC, Lead Poisoning, National Safety Council, 2009).

Copper is a transition metal which belongs to period 4 and group IB of the periodic table with atomic

number 29, atomic weight 63.5, density 8.96 g cm^{-3}, melting point 1083^{o}C and boiling point 2595^{o}C. The metal's average density and concentrations in crustal rocks are 8.1 ×103 kgm−3 and 55mg kg−1, respectively (Davies and Jones, 1988) Copper is the third most used metal in the world (VCI, Copper history/Future, Van Commodities Inc., 2011). Copper is an essential micronutrient required in the growth of both plants and animals. In humans, it helps in the production of blood haemoglobin. In plants, Cu is especially important in seed production, disease resistance and regulation of water. Copper is indeed essential, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation. Copper normally occurs in drinking water from Cu pipes, as well as from additives designed to control algal growth. While Cu's interaction with the environment is complex, research shows that most Cu introduced into the environment is, or rapidly becomes, stable and results in a form which does not pose a risk to the environment. In fact, unlike some man-made materials, Cu is not magnified in the body or bioaccumulated in the food chain. In the soil, Cu strongly complexes to the organic implying that only a small fraction of copper will be found in solution as ionic copper, Cu(II). The solubility of Cu is drastically increased at pH 5.5 (Mart´ınez and Motto, 2000), which is rather close to the ideal farmland pH of 6.0–6.5 (Eriksson, Andersson, and Andersson, 1997).

Due to their properties such as toxicity, persistence and non-biodegradation, contamination with metals has become a serious and widespread environmental threat, particularly in urban areas (Yang-Guang *et al.,* 2016). The problem of heavy metals stems out not only from their toxic properties but also from their ability to accumulate in the body, as it is a case with all elements listed above. At low levels of exposure to these elements, clinical signs do not manifest immediately and their effects can be observed only at the physiological or biochemical level (Wojciechowska-Mazurek *et al.,* 2008).

MATERIALS AND METHODS

The pesticide used for the present study is Bifenthrin. It is collected from the local market, and it is handled with care.

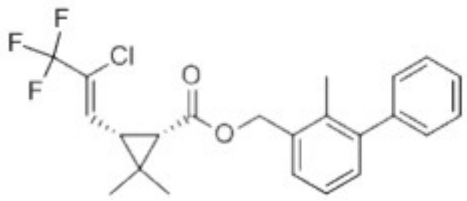
Common Name : Bifenthrin

IUPAC Name : 2-methyl-3-phenylbenzyl (1RS)-cis-3-(2-chloro-3,3,3- trifluoroprop-1-enyl)- 2,2-dimethylcyclopropanecarboxylate

C.A. Name : (2-methyl[1,1'-biphenyl]-3-yl)methyl (1R,3R)-rel-3-[(1Z)- 2-chloro-3,3,3-trifluoro-1-propenyl]-2,2- dimethylcyclopropanecarboxylate

Chemical Family : Pyrethroid

Structural Formula :



Empirical formula : $C_{23}H_{22}ClF_3O_2$

Molecular Weight : 422.88

C.A.S. No. : 82657-04-3

Physical State : Off-white waxy solid

Melting Point : 79.6

Odour : Weak aromatic

Density : 1.26 g/l at 20ºC

Vapour Pressure : 0.0178 mPa at 25ºC

Flash Point : 151ºC

Explosion Hazard : Not explosive or oxidizing

Solubility in water : 0.001 mg/l at 20 ºC

Stability : Stable pH 5 to pH 9 at 25ºC. Hydrolysis occurs at elevated temperatures e.g. DT_{50} 20 mins at pH 4, 90ºC.

Collection of soil:

Garden soil was collected from the college campus and two different agriculture fields. Soil was collected from the Vedanatham village, Tuticorin for the purpose of isolation of pesticides resistant fungi and bacteria using enrichment technique, with varying concentration of pesticides (Bifenthrin) in the medium. The collected samples were brought to the laboratory for analysis. Before analysis, the collected samples were air dried, ground and passed through a 2mm pore size sieve and stored in sealed plastic bags at room temperature. The pysico chemical characters of the soils were analysed using standard methods. The duplicate soil samples were used for remaining experimentation.

Soil analysis of field soil and garden soil:

Moisture content, Soil pH, Organic matter and Bulk density of soils are analyzed by respective procedures.

Isolation of Bifenthrin resistant Fungi and Bacteria:

10g of garden soil, black and red soil were suspended in 250ml nutrient medium supplemented with 50 mg/L Bifenthrin and incubated at 30ºC on plate form shaker at 200 rpm. After 5 days of incubation, 5ml culture was used to inoculate into the nutrient medium containing 100mg/L of Bifenthrin. Subsequently five rounds of enrichment process were carried out in nutrient medium supplemented with higher concentration of Bifenthrin (150 mg/L, 200 mg/L and 250 mg/L). The enrichment culture technique was used for the isolation of fungal and bacterial strains capable of utilizing Bifenthrin as a sole source of carbon

and energy. Enriched medium was serially diluted and 0.1ml of aliquots was plated on nutrient agar plates supplemented with 250 mg/L Bifenthrin for the isolation of resistant fungal cultures.

The petridishes were observed on the 6th day to 9th day for the appearance of resistant colonies. Morphologically different types of colonies were streaked for their purification on nutrient agar plates containing 250 mg/ L Bifenthrin.

Purification of fungal and bacterial isolates:

The isolated colonies in the 5th round of experiment (pesticides bifenthrin resistant fungi and bacteria) were picked up with the help of sterilized wire loop and were streaked on potato dextrose/nutrient agar medium containing 250 mg/L of bifenthrin Each isolated strain was streaked at least 3 to 4 times on agar plates containing 250 mg/L bifenthrin for purification. The isolated and purified fungal and bacterial strains were stored under refrigeration after preparing slants.

Identification of fungal and bacterial isolates:

The identification of fungi and bacteria exhibiting the activity of bifenthrin degradation was carried out based on its colony characters and microscopic observations.

Enzyme production:

Amylase:

100ml of potato dextrose/nutrient agar medium is mixed with 0.5g starch and inoculated the medium with fungal and bacterial growth in 150mg/ml bifenthrin. After the growing of fungi potassium iodine 0.25g/12.5 ml and iodine 0.125g/12.5 ml were added and excess of the solution was removed. After the observation of enzyme activity the zone will form.

Cellulase:

For the production of cellulase, required materials are Berg's medium,CMC agar medium, Whatman no.1 filter paper,0.1% aqueous Cango red, 1M NaCl.

Composition of Berg's medium

Sodium nitrate	2 g
Magnesium sulphate	0.5 g
Dipotassium hydrogen phosphate	0.05 g
Ferrous sulphate	0.01 g
Calcium chloride	0.02 g
Manganese sulphate	0.02 g
Agar	20 g
Distilled water	100 ml
pH	7 g

Composition of CMC agar medium

Carboxy methyl cellulose	1 g

Procedure

Berg's agar plates are prepared, allowed to solidify and labelled 0.1 ml of samples are spread over Berg's agar plates. Then the plates are overlayed with cellulose paper (Whatman no.1) and incubated overnight. Yellow colour colonies are observed and the filter paper are subcultured in CMC agar plates and incubated for 24 hours. After incubation, 0.1% aqueous Congo red is added and left for 20 minutes. The plates are washed with 1M sodium chloride and observing a zone of inhibition around the colonies.

Study on effect of temperature on pesticide resistance fungal growth:

To study the stability of bifenthrin resistant fungal and bacterial isolates, an experiment was conducted in an Erlenmeyer flask containing bifenthrin in 100 ml nutrient/ potato dextrose agar . After sterilization by autoclaving, the flasks were cooled and inoculated with the respective fungal and bacterial cultures and

maintained at different temperatures (15°C, 35°C, 45°C and 55°C). After 8 days, 100ml of culture was drawn and centrifuged at 3000rpm for 10 minutes and OD was taken. The optical density was taken at 600nm using UV – spectrophotometer. Non inoculated control was taken as a blank.

Determination of Heavy metals in sample soils:

1000ppm stock standard solutions of the heavy metals Cu, Cd, and Pb were used to prepare calibration standard and spiking standard solutions. Double distilled water was used throughout the study. The glassware and polyethylene containers used for analysis were washed with tap water, then soaked overnight in 10% (v/v) HNO_3 solution and rinsed several times with double distilled water to eliminate absorbance due to detergent.

Preparation of sediment extract:

Wet digestion method was used for digestion of the soil samples. 0.5 g of each of the air-dried, ground, and sieved soil samples was accurately weighed in a digestion tube. Few drops of distilled water and 2ml of concentrated Nitric acid and 2ml of Perchloric acid were added. Thoroughly agitated, contents were heated gently on hot plate and cooled. Then 21ml of diluted sulphuric acid it was added and boiled for 15 minutes. After cooling, filtered with whatman paper no.44 and diluted with 250 ml of distilled water.

Analysis of heavy metal:

The instrument was calibrated using calibration blank and three series of working standard solutions of each metal to be analyzed. The digested samples were determined for the concentrations of heavy metals (Cu, Cd, and Pb) using flame atomic absorption spectrophotometer Final concentrations of the metals in the soil samples were calculated using the following formula,

$$\text{Concentration (mg/kg)} = \frac{\text{Concentration (mg/L)} \times V}{W}$$

where V = Final volume (50 ml) of solution, and M = Initial weight (0.5 g) of sample measured.

RESULTS AND DISCUSSION

Physico-chemical identification of soil

In the present study, garden soil contaminated with pesticides and its physico chemical properties such as soil pH, moisture content, bulk density and organic matter were estimated. The selected garden soil was used for the purpose of isolation of pesticides resistant bacteria using enrichment technique, with varying concentration of bifenthrin in the medium**.**

The selected soils for isolation of pesticides resistant fungi and bacteria were analysed for their physico chemical characteristics and the results obtained were presented (Table -1).

Selection of pesticides resistant fungi and bacteria through enrichment technique

The selected soils were subjected to enrichment technique with pesticide bifenthrin. At each round of enrichment process, the fungal and bacterial species were observed from bifenthrin contaminated soil by serial dilution method. Different fungal and bacterial colonies were observed in potato dextrose/nutrient agar medium. In the first round of enrichment (50mg/ ml) in potato dextrose/ nutrient agar medium, showed many types of fungal colonies and bacterial colonies. In the second round of enrichment experiment, the fungi and bacteria, highly sensitive to bifenthrin were not grown. Subsequent rounds showed the reduction in the number of colonies. At the end of fifth round of enrichment technique, only three isolated colonies of fungi and bacteria were detected in each type of soils. These six colonies were considered as pesticide resistant fungi and bacteria. Plate 1- 3 showed the bifenthrin resistant bacteria and plate 4-6 showed bifenthrin resistant fungi.

Purification of pesticide resistant fungi and bacteria

Each isolated strain in the 5^{th} round of enrichment technique was pure cultured by streaking at least 3 to 4 times on potato dextrose/nutrient agar plates with the wire loop streaking method for purification. The results obtained were shown in (plate 7-12).

Identification of fungi and characterization of bifenthrin resistant fungi and bacteria

Based on the colony characterization and microscopic observation, the fungal colonies in 5th round of enrichment technique were identified as *Aspergillus niger , Aspergillus flavus* and *Aspergillus ochraceus* (plate 13-15). Bacterial colonies identified as *Aeromonas veroni, Pseudomonas aeruginosa* and *Pseudomonas putida.* Macroscopically *Aspergillus niger* fungus can be identified growing on substrates producing colonies of yellow to white hyphae, turning black with the formation of conidia. Microscopically, *Aspergillus niger* can be identified by its hyaline, septate hyphae. Asexual conidiophores can be identified by being long and globose tip with hymenial layer of structures, each " ejecting" its own spore. *Aspergillus flavus* colonies coloured greyish green but sometimes pure yellow becoming greenish in age and scleotia produced by about 50 % of isolates at first white, becoming deep reddish brown, sclerotia spherical usually 400-800μm in diameter. Structures bearing conidia 400 μm to 1mm or more long. *Aspergillus ochraceus* colonies are light to golden yellow coloured, sclerotia sometimes produced, white when young, later pink to purple, colonies are 40 -50 mm in diameter plane.

Effect of temperature on the growth of bacterial and fungal isolates in pesticide added PDA/nutrient medium

The growth response of fungal and bacterial cultures in nutrient broth amended with bifenthrin at room temperature and under shaking condition was found to be varying significantly.The growth response was monitored by measuring the absorbance of cultures at 600 nm for a period of 15 days. Majority of the growth was found to be maximal in 8th day of incubation. For bifenthrin, the highest growth was recorded in 8th day of incubation (0.441). The OD values ranges from 0.219 to 0.560 and finally decreased in 16th day of incubation (0.450).Growth rates increased in *Pseudomonas aeruginosa* compared with other bacteria. Similarly bifenthrin amended fungi had OD values from 0.392 to 0.492, 0.402 to 0.559. OD values decreased in 16th day of incubation to 0.323, 0.492 and 0.559 respectively shown in (Table 2). Growth rates increased in *Aspergillus flavus* compared to *Aspergillus niger and Aspergillus ochraceus*.

Regarding the effect of temperature on growth rates, the isolates showed better growth, 2.9 mm at 15ºC on 7th day. In fact, some of the species used in this study have optimal growth at 35ºC, with growth rates at optimum conditions of 7.0 ± 0.5 mm per day But, because 15ºC is environmentally more relevant to U.K. and other European countries, where low temperature in soil can be a limiting factor to microbial degradation of soil contaminants the subsequent screening test, to evaluate fungal tolerance to pesticides, was carried out at 15ºC. Lower growth rates do not necessarily imply lower enzyme production,important in the context of degradation of xenobiotics, but the fungi has to grow well for colonisation establishment. The direct objective measurement of fungal growth in soil is difficult since the hyphae stick to the solid substrate therefore the quantification of fungal colonisation must examine different parameters on metabolic activity and enzyme activity.

Enzyme production

The enzyme activity of fungi and bacteria analysis were carried out.

Amylase

Fungi and bacteria tested for amylase activity based on starch iodine method indicated that bacteria produce more amylase than fungi and it was identified by the decolourization of iodine stain. (plate -15-20) (fig-1). *Pseudomonas aeruginosa* showed maximum amylase activity than other bacteria and fungi.

Cellulase

Fungi and bacteria tested for cellulase activity based on the congo red method indicated that fungi produce more cellulase than bacteria and it was identified by the decolouration of congo red stain (plate-21-26). (fig -1) *Aspergillus niger* showed maximum cellulase activity than other bacteria and fungi.

Enzyme activities are involved in processes important to soil function, such as organic matter decomposition and synthesis, nutrient cycling and decomposition of xenobiotics. In the current study, cellulase and amylase activities were assessed, in order to study the response of fungal and bacterial inoculants to a mixture of pesticides in soil. This group of enzymes should represent the responses of a diverse microbial assemblage (fungal inoculants and native soil flora) to a wide range of substrate types and more importantly to

the contaminating pesticides. For biodegradation to take place, the fungal and bacterial inoculants introduced in the soil and/or the native soil microflora must be able to produce degradative enzymes, that remain active in the contaminated soil.

Concentraction of heavy metals in the soil samples

The concentrations of heavy metals in the soil samples are shown in figure 1-4 and table 3-5.The data revealed that all the analyzed metals accumulated by the soil at different concentrations. The result in figure 1 revealed that the concentration of copper (Cu) ranged between 64mg/kg to 65mg/kg. The concentration of copper was high in the field red soil. Cadmium (Cd) was the abundant element in the soil samples studied, shown in figure 2. The cadmium content of soil in all the sampling sites were almost similar, that is 72 mg/kg to 73mg/kg. The high level of cadmium might be due to the use of cadmium-containing phosphate fertilizers and contamination from cadmium-containing dusts. Results of lead (Pb) concentrations ranged from 62 mg/kg to 63mg/kg, that was shown in figure 3. The relatively high levels of lead might have resulted from accumulation of lead through air pollution such as automobile exhaust fumes and from some pesticides, such as lead arsenates applied during cultivation. Comparative study of heavy metals was shown in figure 4. All the metals were relatively high in the field red soil.

SUMMARY AND CONCLUSION

Persistant organic pollutants are becoming an increasing global concern. Developed nations have the capacity to find alternatives and high cost cleaning up treatments for these harmful pollutants. Government of developed nation already banned several of these persistent pesticides as a cleanup mechanism. But developing nations like India still rely mainly on clean hazardous chemicals for the pest control. There is no proper regulatory mechanism to use these pesticides leading to potential harm to the environment and human health as well. Hence, there must be some assistance to implement alternatives to clean up the contamination that these chemicals caused.

Bifenthrin is the organophosphorus pesticide identified by the UN treaty as the potential hazardous pesticide of greater concern, as it is capable of persisting in the environment quite a long time and can cause so many metabolic disorders to human being. However, chemicals are under constant use by farmers of developing countries, particularly in India. Remediation projects have been conducted at numerous pesticides contaminated soil. Incineration and low temperature thermal desorption are proven and frequently used methods for the remediation of these sites that gaining no positive results. So, the bioremediation and phytoremediation are the innovative alternatives that are gaining support as many bioremediation strategies have been successfully reported. Many of these successful phytoremediation technologies have been employed at different sites contaminated with different pesticides such as atrazine, glyphosate, cypermythrin. Keeping this in mind the present study was aimed to understand bioremediation potential of selected fungi and bacteria and their enyzme activities to remediate toxicns. The study involves the collection of literature survey related to pesticides usage of local farmers and the extent of contamination of these fields with persistant pesticides such as bifenthrin. Before the bioremediation projects was begun, all the soil specific phytochemical parameters were surveyed and that revealed the continuous application of these pesticides and other chemical fertilizers rendered the soil unproductiveness for further cultivation. The array of literature also indicated that agricultural land of Thoothukudi district was contaminated with pesticides like bifenthrin at the range between 50mg/L to 250 mg/L.

Hence the bioremediation potential study under lab conditions were done by using these pesticides at the concentration between 50mg/L and 250 mg/L. Further investigation was started with isolation of resistant fungal and bacterial strains from soils supplemented with pesticide such as bifenthrin at different concentrations that coincides to the level of contamination in the natural field.

This trial involves enrichment technique. After 5 rounds of enrichment technique, totally 3 different fungal strains and 3 different bacterial strains resistant to selected pesticide were reported. The identified fungi were *Aspergillus niger, Aspergillus flavus* and *Aspergillus ochraceus* and bacterial strains were *Aeromonas veroni, Pseudomonas aeruginosa* and *Pseudomonas putida* .

In order to understand the physical condition, that is the themperature effect on growth of these fungi at different incubation temperatures such as 15ºC, 35ºC, 45ºC and 55ºC were selected, and the OD of the culture broth was measured at 600 nm, after 48 hours of incubation at respective temperatures. The highest growth rate was seen in *Pseudomonas aerugionsa* bacteria and *Aspergillus flavus* fungi. Of the six strains, fungi and bacteria tested for amylase activity based on starch iodine method indicated that all bacterial strains showed more activity than the fungal strains as their zones were measured.Of the six strains, fungi and bacteria tested for cellulase activity based on the congo red method indicated that fungal strains showed more activity than the bacterial strains as zone were measured.

Enzyme activities are involved in processes important to soil function, such as organic matter decomposition and synthesis, nutrient cycling and decomposition of xenobiotics. In the current study, cellulase and amylase activities were assessed, in order to study the response of fungal inoculants to a mixture of pesticides in soil. This group of enzymes should represent the responses of a diverse microbial assemblage (fungal inoculants and native soil flora) to a wide range of substrate types and more importantly to the contaminating pesticides. For biodegradation to take place, the fungal inoculants introduced in the soil and/or the native soil microflora must be able to produce degradative enzymes, that remain active in the contaminated soil.

The concentrations of heavy metals in soil samples have been determined. The study indicated that the soils served as the potential source of the heavy metals like Cu, Cd, and Pb in the environment. The

significance of increase in the elemental concentrations may be due to application of various types of pesticides and fertilizers in the vegetable farming areas. The results also showed that the level of contaminated soils by the heavy metals is not so high at present. Therefore, the soils studied were not harm for the cultivation and other agricultural purposes, so there is no serious implication for health hazard.

By conclusion, Bifenthrin pesticide contaminated garden soil, field red soil and black soil were bioremediated by fungal and bacterial strains. Comparatively out of six bacterial and fungal strains the bacteria P*seudomonas aeruginosa* showed maximum growth rate when the temperature was increased to 55ºC and also *Pseudomonas aerugionosa* showed maximum activity of amylase by starch iodine method. So when compared to fungi, bacteria served as the good bioremediating sources for cleaning up the environmental pollutants in the soil.

Table - 1 SOIL PROPERTIES OF SELECTED SOIL SAMPLES

PARAMETERS	GARDEN SOIL	BLACK SOIL	RED SOIL
SOIL pH	6.90 ± 0.3	6.96±0.4	6.77 ±02
MOISTURE CONTENT (%)	11.35 ±0.3	16.6±0.2	11 ± 0.4
BULK DENSITY (g/cm^3)	0.177±0.1	0.166 ±0.2	178± 0.2
ORGANIC MATTER (%)	4.90±0.2	4.20±0.3	5.03 ± 0.2

Mean of three replicates ± SD

Table -2 EFFECT OF TEMPERATURE ON GROWTH RATE OF BACTERIAL AND FUNGAL ISOLATES (ºC)

TEMPERATURE	15ºC	35ºC	45ºC	55º
Aeromonas veroni	0.219 ±0.3	0.280± 0.3	0.340 ±0.4	0.441 ± 0.3
Pseudomonas aeruginosa	0.392 ±0.2	0.559±0.5	0.492±0.4	0.323±0.5
Pseudomonas putida	0.196 ±0.3	0.550 ± 0.3	0.450 ±0.6	0.291 ±0.5
Aspergillus niger	0.312 ±0.4	0.543 ±0.5	0.329 ±0.6	0.152 ±0.3
Aspergillus flavus	0.326 ±0.3	0.586 ±0.4	0.496 ±0.6	0.288± 0.4
Aspergillus ochraceus	0.309 ±0.5	0.453 ±0.2	0.400 ±0.3	0.191±0.5

Mean of three replicates ± SD

Table -3 HEAVY METAL ANALYSIS IN SELECTED SOIL SAMPLES - COPPER

Samples	2ppm	4ppm	6ppm	Mean value	(mg/kg)
Black soil	0.640	0.652	0.656	0.649 ±0.2	64.9
Garden soil	0.657	0.644	0.654	0.651 ± 0.4	65.1
Red soil	0.648	0.690	0.660	0.653 ± 0.3	65.3

Table -4 HEAVY METAL ANALYSIS IN SELECTED SOIL SAMPLES - CADMIUM

Samples	2ppm	4ppm	6ppm	Mean value	(mg/kg)
Black soil	0.729	0.731	0.728	0.729±0.2	72.9
Garden soil	0.727	0.735	0.729	0.730± 0.3	73
Red soil	0.732	0.727	0.737	0.732±0.4	73.2

Table -5 HEAVY METAL ANALYSIS IN SELETED SOIL SAMPLES - LEAD

Samples	2ppm	4ppm	6ppm	Mean value	(mg/kg)
Black soil	0.630	0.632	0.632	0.627	62.7
Garden soil	0.625	0.632	0.622	0.626	62.6
Red soil	0.629	0.635	0.6224	0.629	62.9

Fig-1 Enzymatic activity of selected microorganisms

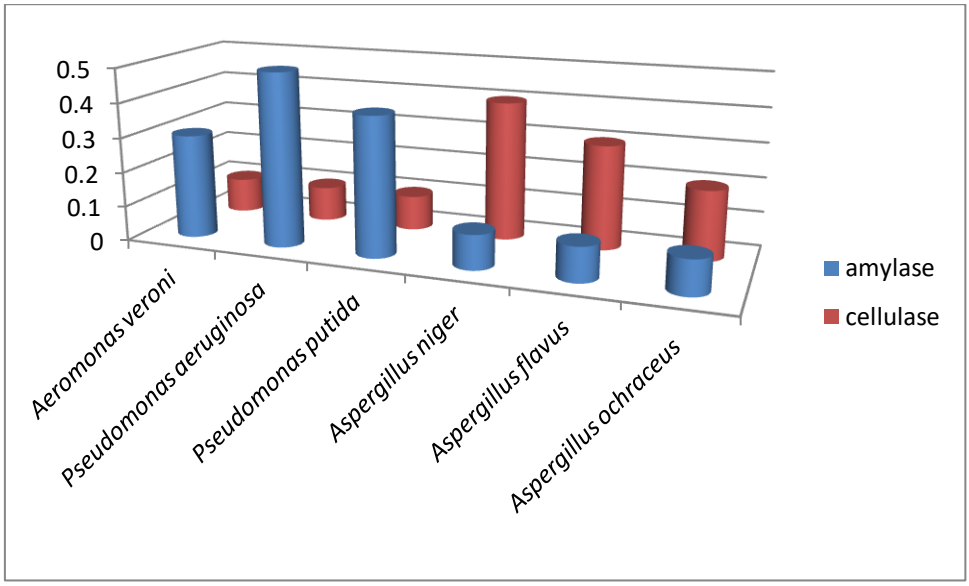
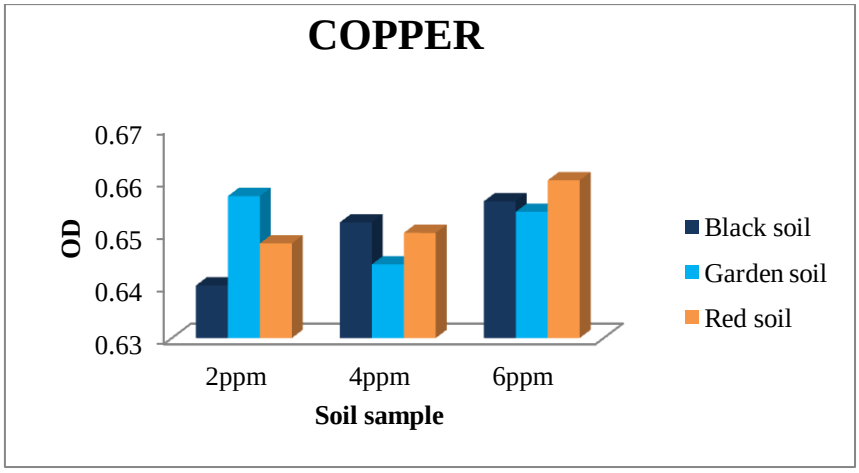
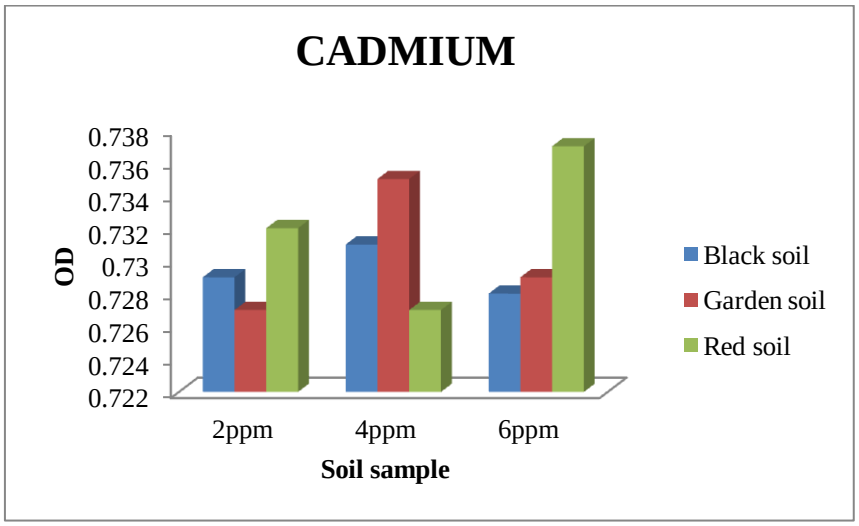


Fig -2 Heavy metal analysis in selected soil samples





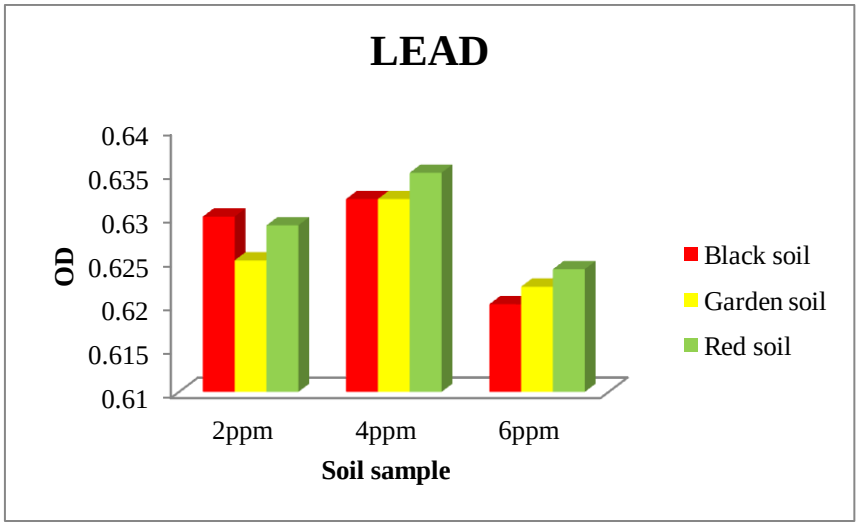


Fig -3 Heavy metal analysis in selected soil samples

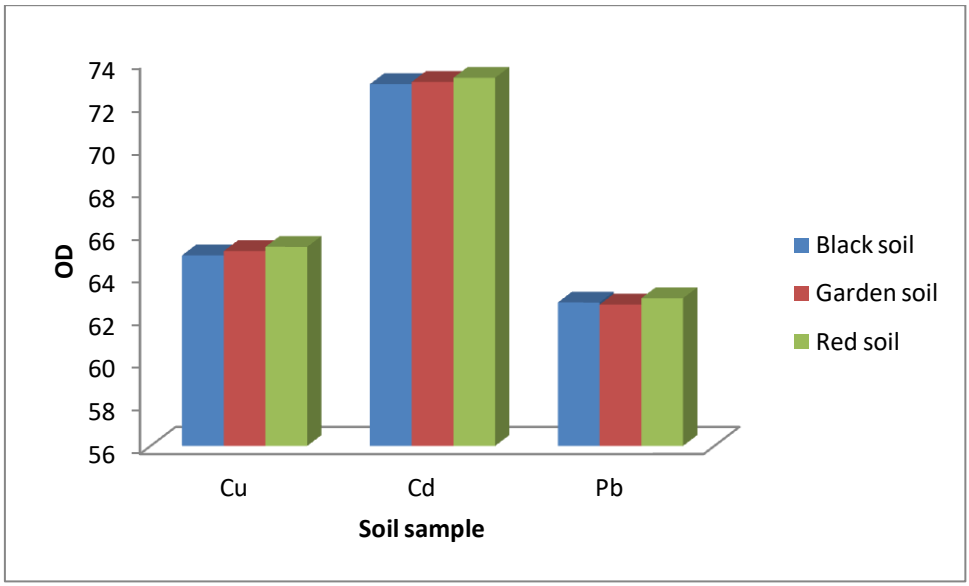
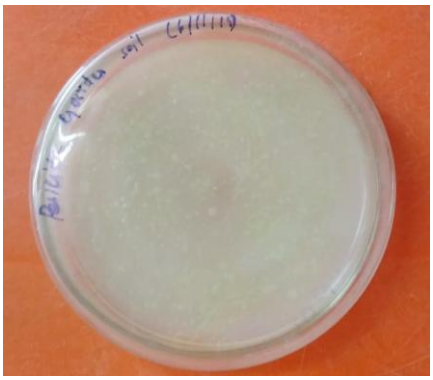


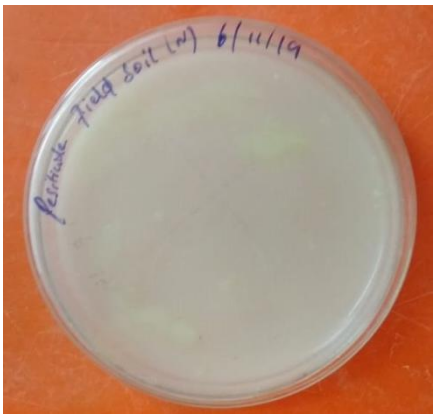
Plate -1

Isolation of microorganisms by Enrichment technique



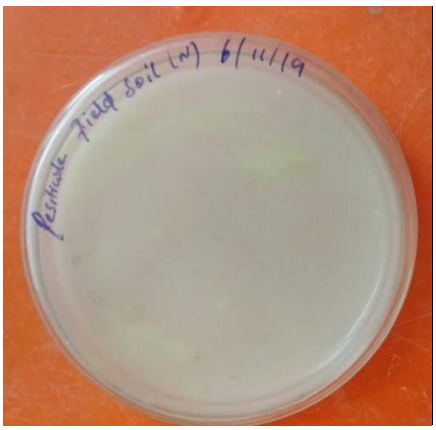
250mg/lit garden soil (bacteria)

250mg/lit garden soil (fungi)

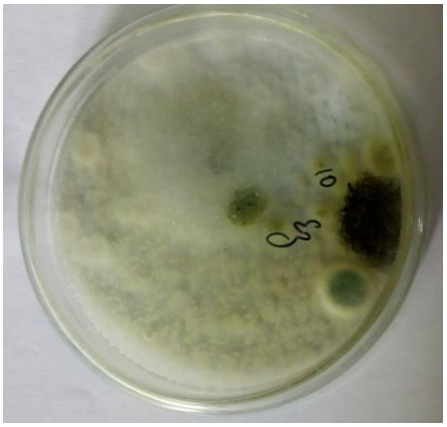


250mg/lit field black soil (bacteria)

250mg/lit field black soil (fungi)



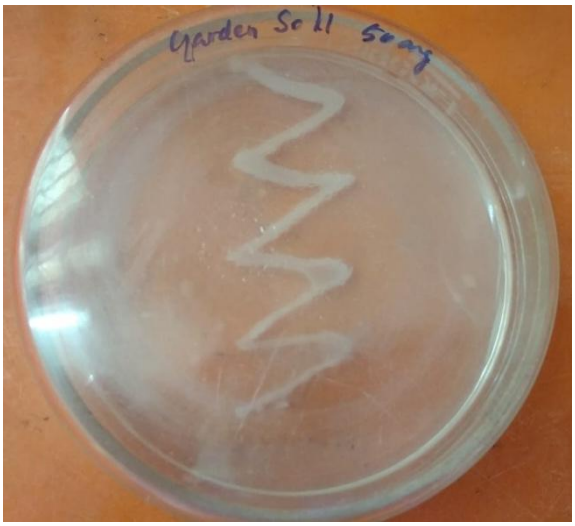
250 mg/lit field red soil (bacteria)



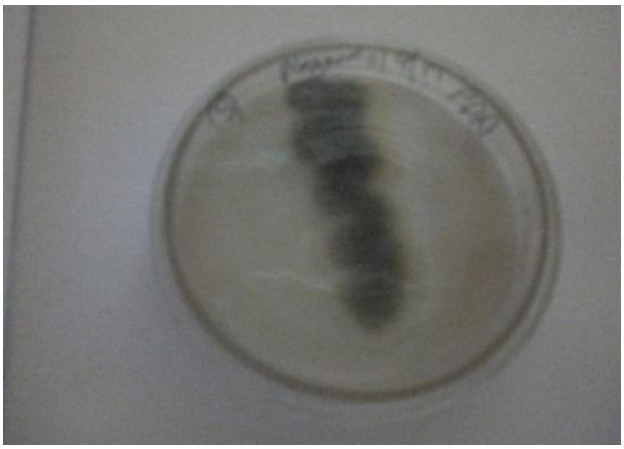
250mg/lit field red soil (fungi)

Plate-2

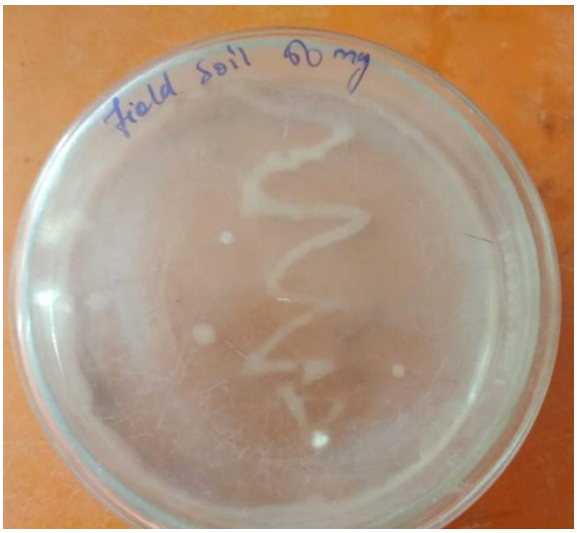
Purification of microorganisms by Streak plate method



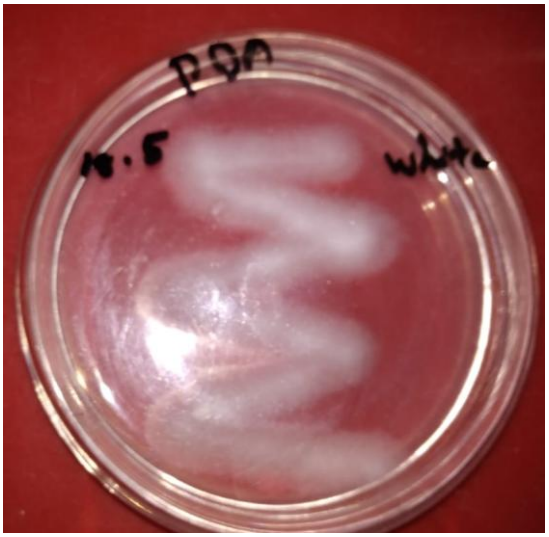
Purified garden soil bacteria



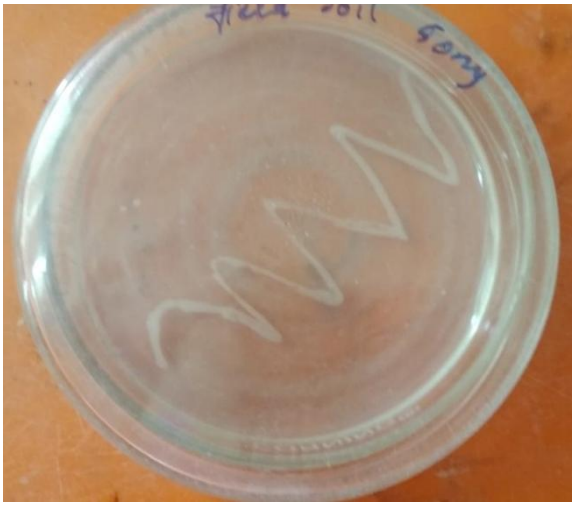
Purified field black soil fungi



Purified field black soil bacteria



Purified field black soil fungi

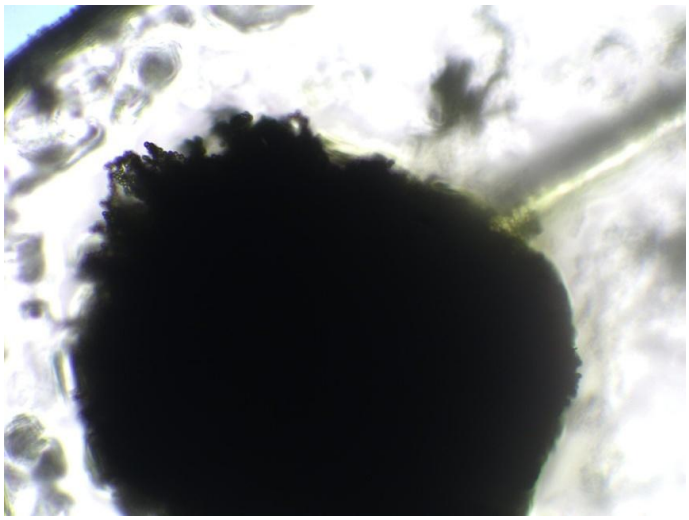


Purified field red soil bacteria

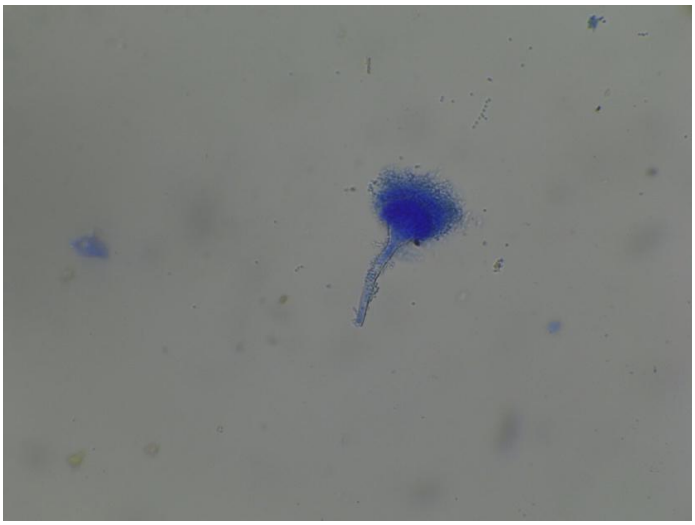
Purified field red soil fungi

Plate -3

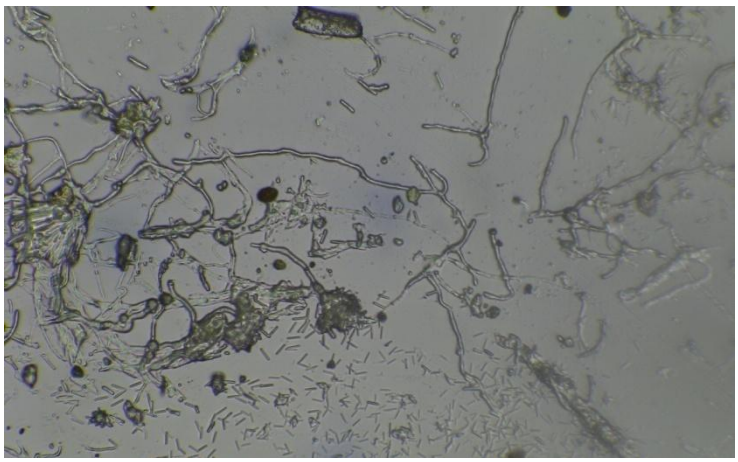
Microscopic observation of microorganisms



Microscopic observation of *Aspergillus niger*



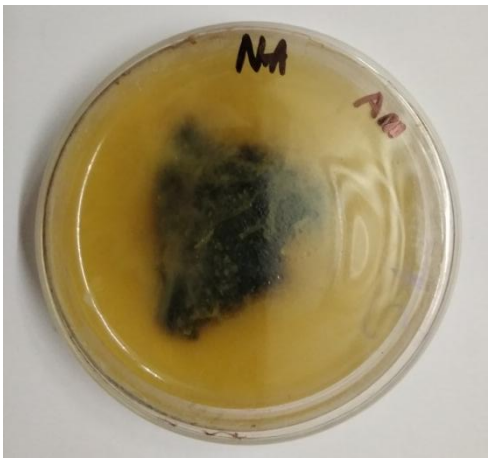
Microscopic observation of *Aspergillus flavus*



Microscopic observation of *Aspergillus ochraceus*

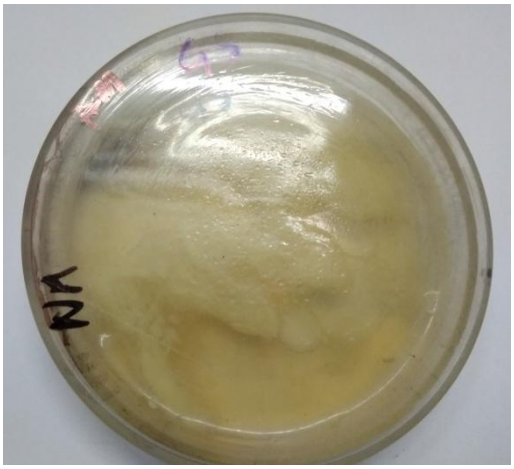
Plate- 4

Amylase activity of microorganisms by Starch iodine method



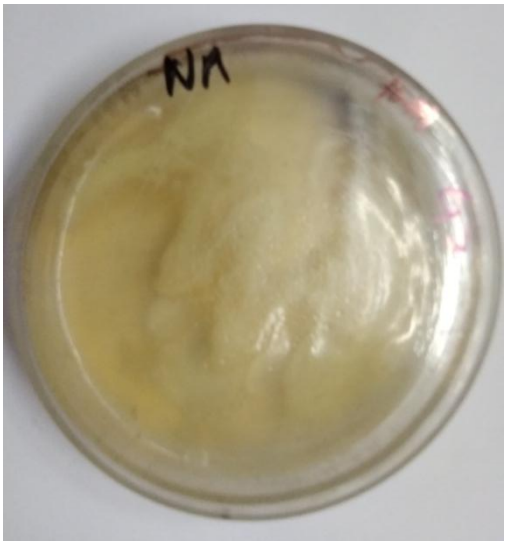
Amylase activity of *Pseudomonas putida*

Amylase activity of *Aspergillus niger*



Amylase activity of *Aeromonas veroni*

Amylase activity of *Aspergillus flavus*

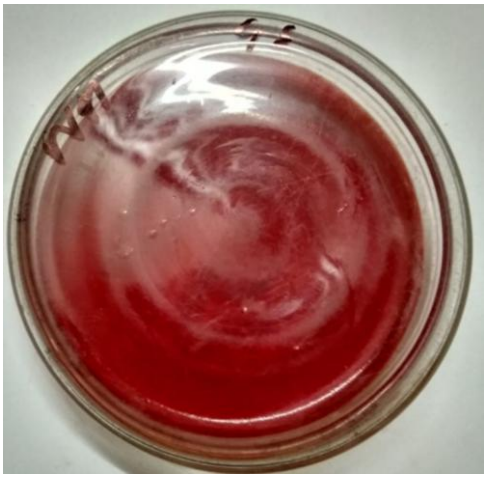


Amylase activity of *Pseudomonas aeruginosa*

Amylase activity of *Aspergillus ochraceus*

Plate -5

Cellulase activity of microorganisms by Congo red method

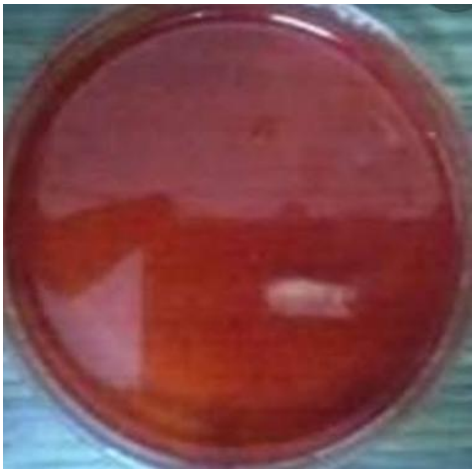


Cellulase activity of *Pseudomonas putida*

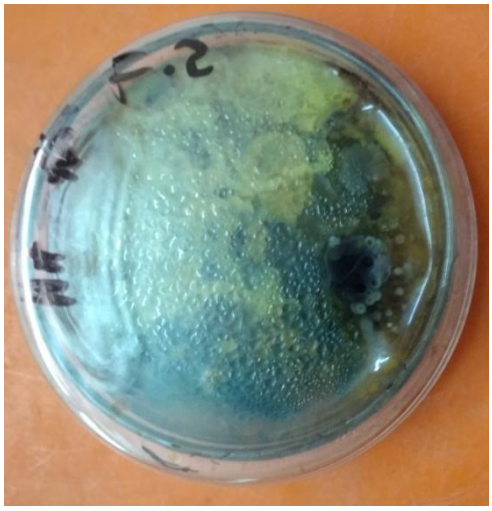
Cellulase activity of *Aspergillus niger*

Cellulase activity of *Aeromonas veroni*

Cellulase activity of *Aspergillus flavus*



Cellulase activity of *Pseudomonas aeruginosa*



Cellulase activity of *Aspergillus ochraceus*

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PLEUROTUS CULTIVATION: A SUSTAINABLE WAY FOR BIOTRANSFORMATION OF AGROWASTE TO POTENTIAL NUTRITIOUS FOOD AND FERTILIZER

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By

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CERTIFICATE

It is certified that this short term project work entitled **"Pleurotus cultivation: A sustainable way for biotransformation of agrowaste to potential nutritious food and fertilizer"** submitted by **A. Selvaananthi** in partial fulfillment of **M.Phil. degree**, in Botany to St. Mary's College (Autonomous), Thoothukudi affiliated to Manonmaniam Sundaranar University, Tirunelveli is based on the results of studies carried out by her under my guidance and supervision. It is further certified that it has not been submitted for the award of any degree, diploma, associateship, fellowship of any University/Institution.

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DECLARATION

I do hereby declare that this work has been originally carried out by me under the guidance and supervision of **Dr. Sr. A. Arockia Jenecius Alphonse,** Assistant Professor of Botany, St. Mary's College (Autonomous), Thoothukudi and this work has not been submitted elsewhere for the award of any other degree.

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A. SELVAANANTHI

CONTENTS

INTRODUCTION

INTRODUCTION

India has diverse agro-climatic zones which are suitable for cultivating wide range of plants including food crops, cash crops and horticultural products. Burgeoning demand for food throughout the world has led to an exponential increase in food production as anywhere in the world all over India. The advancement of agricultural production has undoubtedly resulted in increased amount of agricultural waste and agro-industrial waste. A significant growth of agricultural waste is prone to occur globally if developing countries continue to step up farming systems. This organic waste should be handled carefully in a sustainable way to avoid unwanted environmental side effects. Agricultural waste also termed as crop residue which includes field residues as well as processed residues. Some examples: straw from cereals, dry leaves, saw dust and all the existing portions after harvesting the yield etc. In most of the states of India, crop residues are mainly utilized for animal feed.

Many countries leverage crop residues produced by farming practices in different directions. It has been used in a processed or unprocessed form depending on the desired application. Potential alternatives include livestock feed, composting, bio-energy production and deployment in other extended farming activities like cultivation of mushrooms **(Monforti *et al.*, 2013; Hayashi *et al.*, 2014; Lohan *et al.*, 2018).** Several nations including Japan, China, Nepal, Malaysia, Nigeria, Indonesia, Thailand and Philippines are using their agricultural waste to develop bio-energy and fertilizers **(Lohan *et al.*, 2018).**

Champignon is commonly known as an edible mushroom which is a popular food due to its unique taste, nutritious value and medicinal properties. Mushroom cultivation has become popular throughout the world. Edible fungi production can greatly enhance sustainability, economic strength of the farmers. Diversification in agriculture sector is inevitable because of some key factors like population growth, food scarcity, poverty and malnutrition among developing and underdeveloped countries. Mushroom cultivation is an ideal method which posses unique advantages than other waste management technologies. It helps the farmers to increase their income effectively by utilizing their own agricultural land waste. This kind of management has drawn more and more attention because of the nutritious output from waste. Mushroom cultivation can help to mitigate hunger and improve livelihoods by providing a fast-growing nutritious food supply and a stable source of employment and wealth **(Rachna *et al*., 2013).**

Nearly 355 million tones of crop residues are produced every year. In this, about 170 million is left out for burning and manure preparation. If India utilizes one percent of these waste, it will become a top mushroom producing country in the world **(Tewari and Pandey, 2002).** Alternative initiatives have previously been demonstrated by scientists and agriculturalists over the last decade to overcome the burning of crop residues, but due to the absence of awareness among growers these initiatives have not yet been completely adopted **(Bhuvaneshwari *et al*., 2019).**

Mushrooms are the spore-bearing fruiting bodies of matured mycelium typically formed above the soil or on its substrate. Greeks and Romans have taken mushrooms as food since ancient times. Chinese considered mushroom as an elixir of life and Romans regarded it as food of God **(Bashir *et al*., 2014).** Mushroom belongs

to the class Basidomycota of kingdom Fungi because of unique fungal characteristics and development **(Song, 2004).** Mushrooms have drawn attention as a therapeutic food, as a source of drugs and nutritional supplements since they are hyped for their antioxidant, antitumor and antimicrobial properties. In addition to their pharmacological properties, mushrooms have become much more significant in our diet due to its high nutritional value, high protein content and low fat / energy content. **(Khatun *et al*., 2012).**

Different varieties of mushrooms are seen in the world but all are not suitable for human consumption. Edible mushroom includes several wild and cultivated species. Most of the wild mushrooms are poisonous. Consumption of wild varieties without proper identification can cause mild symptoms to death. The colour and structure of mushrooms varies from species to species. Fruiting body of these macro fungi is made up of a network of fungal hyphae. Button mushroom (*Agaricus* spp.), shiitake mushroom (*Lentinus* spp.), oyster mushroom (*Pleurotus* spp.), wood ear mushroom (*Auricula spp.*), winter mushroom (*Flamulina* spp.) and straw mushroom (*Volvariella* spp.) are the most common cultivated species.

Among all the cultivated mushroom, oyster mushroom have many advantages due to rapid mycelial growth, great colonization potential, easy and economical cultivation techniques and suitable for cultivating under different climatic conditions. In addition to this, they are low in calories, sodium, fat and cholesterol, while rich in protein, carbohydrate, fiber, vitamins and minerals. Oyster mushroom and their derivatives are used for medicinal purpose (**Kues and Liu, 2000; Cohen *et al*., 2002**). *Pleurotus* species can be cultivated within a temperature range of 20°- 30°C. It is one of the suitable species to cultivate in tropical and subtropical regions of the world

(Hossain, 2017). Owing to these characteristics, the producers and consumers of mushroom have expanded significantly in recent decades. During the last three decades, the production rate of oyster mushroom has increased nearly 25-fold world widely. **(Chang and Miles, 2004)**.

People are now deeply interest towards natural healthy foods rich in bioactive substances that promotes immune system and reduces the risk of diseases. The up hilling of free radicals results in multiple dangerous diseases. Even though antioxidant defense and repair systems are present in human body to protect it from oxidative damage, these systems are insufficient to prevent the oxidative stresses completely **(Mau *et al*., 2002).** Presence of various bioactive compounds in mushroom can cope with the oxidative damages and thus it can serve as an antioxidant.

Nanotechnology is a fast developing field which has now contributed to exciting transformation in biomedical and engineering in terms of effectiveness, health and economy. Nanobiotechnology is the descendant of nanotechnology and biology. Nanoparticles coated with molecules or bio-moieties are getting attention in nano drug delivery which has unique and targeted uses without affecting the cells in nearby areas of the target. Nanomaterials synthesized in laboratories can enter human body and cause potential health hazards. Therefore, there is a need to establish green chemistry approaches in the nanomaterials synthesis **(Albrecht *et al.,* 2006).** In this respect, synthetic methods based on naturally occurring biomaterials offer better alternative approaches. Silver nanoparticles have enormous capabilities other than its miniature size aspect, which will lead to excellent antimicrobial activity as compared with bulk Ag metal **(Mahendra *et al*., 2009).** Hence, the presence of silver

nanoparticle producing efficiency of mushrooms is added to its pharmacological relevance.

After harvesting the mushrooms the left over residues are called as Spent Mushroom Substrate (SMS). Production of 1kg of mushroom results in 5 – 6 kg of spent mushroom substrate. **(Ma *et al.*, 2014).** Spent mushroom compost has widely used as highly successful manure in India **(Sagar *et al.*, 2009).** Spent Mushroom Substrate of *Agaricus bisporus* is profitably converted to liquid fertilizer **(The Sarnia Journal, 2014). González-Marcos *et al.* (2015)** co-composted *Agaricus* spent substrate with winery sludge and is used for soil application. *Agaricus bisporus* spent substrate effect on soil physicochemical properties has been noted by many authors **(Maher *et al.*, 2000; Medina *et al.*, 2012). Zhu *et al.* (2013)** reported *Pleurotus* spent substrate as a biofertilizer for the cultivation of plants. Extracts and the composts of *Agaricus bisporus* spent substrate proved to be effective against various plant diseases **(Borrero *et al.*, 2013)**. SMS from *Pleurotus* cultivation has been tested against various diseases of cucumber plant **(Parada *et al.*, 2012).**

SCOPE AND OBJECTIVES

SCOPE AND OBJECTIVES

Mushroom, once considered as an haute cuisine, became a common delicacy in recent years after the popularization of simplified mushroom cultivation methods and its large-scale production. Regular intake of mushroom helps to balance the daily protein requirement and also provides essential amino acids, vitamins and minerals. Numerous studies have shown that presence of several bioactive constituents helps to defense against oxidative stress and act as an immune booster. Utilization of agricultural wastes for the cultivation of mushroom can reduce the pollution load on the environment.With the basic knowledge on cultivation techniques, anybody can cultivate mushroom in their home with a minimum initial investment. Therefore, the present study was carried out with the following objectives.

- Preparation of spawn.
- Cultivation of oyster mushroom – *Pleurotus florida* and *Pleurotus ostreatus*.
- Study the effect of paddy straw, sugarcane bagasse and banana leaves on the growth and morphological parameters of *Pleurotus florida* and *Pleurotus ostreatus*.
- Identification of microbial competitors of mushroom cultivation.
- Qualitative analysis of the fruiting bodies of *Pleurotus florida* and *Pleurotus ostreatus*.
- Estimation of proximate composition – carbohydrate, protein, aminoacid and lipid content of *Pleurotus florida* and *Pleurotus ostreatus*.
- Determination of antioxidants – phenol, flavonoid, tannin, vitamin C and vitamin B_2 of *Pleurotus florida* and *Pleurotus ostreatus*.

- Determination of mineral constituent of *Pleurotus florida* and *Pleurotus ostreatus*.
- Study the functional groups and bioactive compounds of *Pleurotus florida* and *Pleurotus ostreatus* by observing FT-IR and GC-MS spectrum.
- Analyze the physico-chemical properties of the substrates.
- Screening of antioxidant activity - free radical scavenging assay (DPPH), ferric ion reducing antioxidant assay and hydrogen peroxide scavenging activity
- Study the antimicrobial efficiency of *Pleurotus florida* and *Pleurotus ostreatus* using disc diffusion method.
- Analyze the effectiveness of *Pleurotus florida* and *Pleurotus ostreatus* in green synthesis of silver nanoparticles.
- Determination of the phytotoxicity of SMS liquid fertilizer on green gram seedlings.
- Study the awareness level among people about mushroom cultivation and nutrient benefits.

REVIEW OF LITERATURE

All over history, mushrooms have brought about diverse reputations, deemed both food and foe. It is a magical delicacy in mycophiles dining. (**Trutmann, 2012**) Edible mushrooms contain plenty of essential nutrients and medicinal properties. They are increasingly being used because of their effective role in boosting human immune system. It represents world's one of the greatest untapped resources of nutritious food material. Cultivation of saprophytic edible mushrooms may be the only emerging affordable biotechnological aid for the processing of lignocellulosic waste which can be used for the production of protein rich food with reduced environmental pollution **(Obodai, 2003)**. Such edible fruiting bodies of mushrooms serve as a valuable source of protein, minerals, vitamins and essential amino acids **(Sadler, 2003).**

The genus *Pleurotus* is a member of gilled fungi which is one of the most widely eaten species commonly known as oyster mushroom. It occupies third place among world mushroom productions after white button and shiitake mushroom. This can be found in both tropical and temperate climates (**Gyorfi and Hajdu, 2007**; **Chang *et al.*, 2004**)

MUSHROOM CULTIVATION USING AGRO ORGANIC WASTE AND ITS PERFORMANCE

India produces about 185 million tons of crop residues every year **(Bellakki and Badonus, 2001).** Agricultural wastes are being disposed by means of land applications, incineration and land filling. Elimination of waste by incineration

cause environmental pollution by gas emission. These million tons of valuable organic agricultural waste can be used to produce nutritious food **(Iscia and Demirer, 2007)** mushroom cultivation.

More than 200 species of mushrooms were collected from the wild and utilized for various traditional medicinal purposes, mainly in the Far East. Approximately 35 species is commercially grown and 20 are cultivated on an industrial scale. The most grown mushroom in the world is *Agaricus bisporus* (button mushroom), followed by *Lentinus edodes* (shiitake), *Pleurotus* spp. (oyster mushrooms), *Auricula auricula* (wood ear mushroom), *Flamulina velutipes* (winter mushroom) and *Volvariella volvacea* (straw mushroom) **(Sánchez, 2004).**

Mushrooms can be cultivated on different substrates which contain lignin and cellulose (**Das and Mukherjee, 2007**). Growth performance of mushroom is depended on the constituent of the substrate **(Iqbal *et al.*, 2005).** Substrates rich in nitrogen and carbohydrate contents are considered as effective a source for mushroom growth (**Khare *et al.*, 2010; Islam *et al.*, 2016; Salama *et al.*, 2016; Singh *et al.*, 2016).** Agricultural residues are one of the highly valuable and renewable lignocellulosic biomass as well as an alternative source for cellulosic materials. Among all other agricultural residues, rice straw has been extensively studied because it is one of the most consumed cereals in the world. Nearly 650 – 975 million tons of paddy straw produced per year all over the world (**Sun *et al.*, 2005; Santos *et al.*, 2017**).

The growth rate of microorganisms and their quantitative and qualitative yield depends on the utilization of nutrients and physicochemical composition of the medium on which it was grown **(Mukhopadhyay *et al.*, 2002).** Rice straw is

composed of approximately 35% cellulose, 18% hemicelluloses and 15% lignin (**Jiang, *et al.*, 2011**). **Singh *et al.* (2014)** reported the presence of 39.04% of cellulose and 16.2 % of lignin content in rice straw. Rice straw is a waste material which constitutes the stem, root and a fraction of the spikes. It is mainly composed of cellulose (32-47%), hemicellulose (19-27%) and lignin (5-24%) (**Karimi *et al.*, 2006**). **Shoaib *et al.* (2018)** reported that rice straw consists of 14.5% of lignin and 34% of cellulose, silica, calcium, phosphorus, potassium, magnesium, sulfur, cobalt, copper and manganese.

Mushroom requires nutritional source like carbon, nitrogen and inorganic compounds during their life cycle. Their growth is mainly depended on some specific carbon sources such as cellulose, hemicellulose and lignin. *Pleurotus* species requires high carbon and less nitrogen source. However, requirement of nutritional source differs from species to species (**Sharma *et al.*, 2013**).

Different agro wastes and agro-industrial wastes such as paddy straw, wheat straw, grasses, coffee cherry husk, sugarcane trash, coir waste, saw dust, malt industry waste, tea-leaf industry waste, wild grasses, weeds, corn cobs, vegetable residues, maize stalks, cotton waste, banana pseudo-stems and leaves are used as an alternate substrate for *Pleurotus* species cultivation (**Singh, 2000; Panneerselvam *et al.*, 2000; Ayyappan *et al.*, 2000; Ohga, 2000; Upadhyay and Verma, 2000; Das *et al.*, 2000; Kumar *et al.*, 2000; Reddy *et al.*, 2001; Hassan *et al.*, 2011; Sardar *et al.*, 2016; Islam *et al.*, 2016; Salama *et al.*, 2016; Siqueira *et al.*, 2016).**

Dey *et al.* (2008) examined the effect of different substrates such as paddy straw, sugarcane bagasse and mustard straw on the production of oyster mushroom by following cylindrical block method. Specific substrates greatly influenced the count

of primordia, fruiting bodies and the yield. The highest number of primordia, fruiting bodies and maximum yield was obtained with sugarcane bagasse while the lowest with mustard straw.

Islam *et al.* (2017) cultivated *Pleurotus ostreatus* upon a number of substrates that specifically influence the pattern of pin head and fruiting body formations. Cotton waste performed as a better substrate and it took minimum days (7.5) to begin pinhead formation followed by *Chenopodium album* and mixture of cotton waste + *Chenopodium album*. More fruiting bodies were developed when cultivation was based on cotton waste, *Chenopodium album* and mixture of cotton waste + *Chenopodium*.

Dubey *et al.* (2019) evaluated the effect of various substrates such as rice straw, wheat straw, banana leaves and sugarcane bagasse on yield performance of oyster mushroom. Maximum yield was reported in paddy straw substrate (1515 g) followed by banana leaves (517.5 g), wheat straw (480 g) and sugarcane bagasse (98.75 g). The maximum stipe length was reached in rice straw followed by banana leaves, wheat straw and sugarcane bagasse. Similarly, cap diameter was also found highest in rice straw followed by wheat straw, banana leaves and sugarcane bagasse.

Bandopadhyay and Chatterjee (2009) observed the highest yield (923.7 g) of *Pleurotus florida* upon combined (paddy straw and water hyacinth) substrates followed by paddy straw alone (698.1 g) and then water hyacinth alone (614.1g). **Salami *et al.* (2017)** cultivated *Pleurotus florida* on four lignocellulosic substrates namely sawdust, corn cobs, oil palm spadix and corn straw. They reported that the highest yield and biological efficiency were in corn cobs substrate (110 g, 55%),

followed by oil palm spadix substrate (76.05 g, 38%) and least on corn straw substrate (63.12 g, 31.56%).

Sardar *et al.* (2016) reported the impact of various agro-industrial wastes (corn cobs, sugarcane bagasse, cotton waste) and their combinations on the yield and quality characteristics of *Pleurotus sajor-caju*. Minimum number of spawn running day was observed in cotton waste (22.45 days) and maximum (26.22 days) in sugarcane bagasse. The highest number of pinhead, fruiting body, yield (259.23 g) and biological efficiency (81.0%) was recorded on cotton waste while minimum in sugarcane bagasse.

Islam *et al.* (2009) observed that *Pleurotus flabellatus* performance on different saw dust. Maximum yield was recorded in mango sawdust (150 g) followed by mahogony (148 g), shiris (146 g), kadom (136 g), jam (114 g), jackfruit (97 g) and coconut sawdust (83g). Various species of *Pleurotus* including *Pleurotus sajor-caju, Pleurotus flabellatus, Pleurotus florida, Pleurotus eous, Pleurotus ostreatus* and *Hypsizygus ulmarius* (Synonyms: *Pleurotus ulmarius*) were studied by **Biswas and Kuiry (2013).** Of these fungi, newly introduced elm oyster mushroom *Hypsizygus ulmarius*is reported to have the highest biological efficiency (156%), followed by *Pleurotus florida* (121.5%), *Pleurotus sajor-caju* (115.5%) and *Pleurotus ostreatus* (103.25%) biological efficiency.

Pleurotus sajor-caju cultivated upon paddy straw, wheat straw, banana leaves, sugarcane bagasse, sugarcane leaves, newspapers, maize stalks and leaves showed considerable difference in their growth performance. Sugarcane required the lowest time for spawn running, primordial initiation and fruiting body formation and it was followed by newspapers, paddy straw, banana leaves and wheat straw. Paddy straw

showed the highest yield and biological efficiency and lowest in maize stalks and leaves (**Hossain, 2017).**

Sharma *et al.* (2013) examined the growth efficiency of *Pleurotus ostreatus* on various substrates and combinations namely rice straw, rice straw + wheat straw, rice straw + paper, sugarcane bagasse and sawdust of alder. Substrates were supplemented with 10% rice bran excluding rice straw and it was considered as the control. In these treatments, rice straw (control) was seen as the best substrate which produced 381.85 g yield with 95.46% biological efficiency followed by rice + wheat straw and rice straw + paper waste.

Patil (2013) conducted an experiment to study the influence of agro waste viz. soybean straw, paddy straw, wheat straw, jowar straw, sunflower stalk and pigeon pea stalk on the productivity and proximate content of *Pleurotus sajor-caju*. Soybean straw showed the highest yield (with 83.00 % B.E.), maximum protein (25.80 %), fat (2.82 %) and ash (7.30 %) content. High moisture content (88.25 %), carbohydrate (58.50 %), crude fiber (7.90 %) was observed on the mushrooms cultivated upon the wheat straw, jowar straw and paddy straw respectively.

Tirkey *et al.* (2017) have found that banana leaf substrates individual as well as in combination proved to be the best for cultivation of *Pleurotus florida* by showing highest yield on banana leaves (915.23 g) with 91 % biological efficiency than other substrates namely wheat straw, cardboard and grass (individuals and combinations).

Neupane *et al.* (2018) have studied the yield of various substrates such as banana leaves, rice straw, wheat straw and saw dust on the production of *Pleurotus florida*. The duration of mycelium colonization, fruitification and harvesting was earlier in banana leaves followed by other substrates and longer in saw

dust. The highest yield was reported in banana leaves (4.76 kg for 5 kg substrate) and lowest in saw dust. Maximum mass of the final substrate was obtained from saw dust (4.56 kg) and minimum in banana leaves (2.12 kg). They conclude that banana leaves are suitable substrate for production of *Pleurotus florida* followed by rice straw among other substrates used.

Sardar *et al.* (2017) reported the effects of various agro-industrial waste materials such as cotton waste (mill droppings), wheat straw, rice straw, corn cobs, sugarcane bagasse and sawdust on nutritional composition of king oyster mushroom (*Pleurotus eryngii*). Mycelium growth and biological efficiency was observed to be the highest on cotton waste compared to other lignocellulosic waste used.

COMPETETOR MOULDS:

In India, most commonly available mushrooms are button mushroom, oyster mushroom, paddy straw mushroom and milky mushroom. Similar to other crops, mushrooms also affected by several biotic and abiotic factors. In the list of biotic factors, fungi, bacteria, viruses, nematodes, insects and mites are mainly responsible for the damage and crop loss. These competitors affect the mushrooms directly or indirectly. Several harmful fungi present in the compost and casing material also affect the spawn run and mushroom growth. On the other side, the fruit body at varying phases of crop development is disturbed by microbes, thereby developing distinct signs of diseases. If this continues, total crop failure occurs based on the stage of infection and environmental conditions. Undesirable growth or development of certain molds can occur and can adversely affect the quality of compost and final mushroom yield **(Sharma, 2007; Chowdhury and Ojha, 2017).**

Microbial contamination in mushroom beds mainly causes yield loss and spread disease to all the beds which are present near to the contaminated bags. Of these pathogens, *Trichoderma harzianum* was stated to be the most dangerous and competing actively with *Pleurotus pulmonarius* and *Pleurotus ostreatus* mycelium (**Shin, 1987**). *Pleurotus florida* was most commonly affected by certain fungal species namely *Aspergillus niger*, *Coprinus* sp., *Sclerotiumrolfsii* and *Penicillium* sp. (**Biswas and Kuiry, 2013**). **Jaivel and Marimuthu (2010)** reported that *Trichoderma*, *Aspergillus* and *Rhizopus* are the predominant microorganisms of *Pleurotus* species especially it infects severely during in summer and spring seasons than autumn and winter.

Biswas (2014) studied the effect of climatic factors on the incidence of competitor moulds and yield of oyster mushroom. The survey revealed the occurrence of *Trichoderma harzianum, Penecillium notatum, Aspergillus niger, Coprinus* sp., *Mucor* sp., *Rhizopus* sp., and *Sclerotium rolfsii* in mushroom beds. Among these competitor moulds *Trichoderma harzianum, Penecillium notatum, Sclerotium rolfsii* and *Coprinus* sp. were found to be the most dominant contaminants of cultivation bags. Microbial contaminants were observed to be minimum during the month of January (2.875 %) and it increases with the fluctuating climatic conditions and reaches the highest during the month of June (32.8%). Pseudomonads cause many diseases in *Agaricus bisporus*. A drippy gill disease was caused by *Pseudomonas agarici* and it showed brown discoloration as recently observed in Italy and the Netherlands (**Cantore and Iacobellis, 2004; Geels *et al*., 2008**). In western countries, mushroom production is mostly affected by *Pseudomonas tolaasii, Lecanicillium fungicola, Trichoderma* sp. and viral particles which count nearly 25% of the total production **(Soković and van Griensven, 2006).**

Shamoli *et al.* (2016) found out the fungal competitors microbes in oyster mushroom spawn packets. Totally nine fungal competitors have been isolated and identified notably *Trichoderma harzianum Trichoderma viride* (Yellow strain), *Trichoderma koningii*, *Trichoderma viride* (Green strain), *Papulaspora byssina*, *Neurospora* sp., *Mucor hiemalis* and *Aspergillus flavus* and *Botryodiplodia theobromae* on the basis of cultural characters, morphological and microscopic features.

Competitors mould belonging to different families has been reported to occur in the substrates which are used for the cultivation of oyster mushroom. Variations in the types of moulds are mainly because of the application of different types of substrate that is agricultural wastes, methods of substrate preparation and the conditions and containers used for cultivation. A number of saprophytic and plant pathogenic fungi present in the substratum are competing with mushroom mycelium for space and food. *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Drechslera bicolor, Fusarium moniliforme, Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Rhizpus stolonifer*, *Sclerotium rolfsii*, *Trichoderma viride* were reported by some researchers **(Sharma *et al*., 2007; Sharma and Kumar, 2011).**

Shah *et al.* (2018) identified the competitor moulds from paddy straw used, spawn bottles, fully spawn-run straw and directly from the fruiting bodies (basidiocarps) of *Aspergillus flavus, Aspergillus niger, Penicillium* sp., *Rhizopus* sp., *Trichoderma harzianum, Alternaria, Cephalosporium, Penicillium mineoluteum, Trichoderma viride, Trichoderma pseudokoninjii* and *Fusarium oxysporum*. Among the isolated species *Trichoderma* sp. and *Penicillium* sp. observed as the most dominant and devastating of oyster mushroom.

Chung *et al.* (1998) reported twenty-nine species and four varieties of slime molds from different edible mushroom cultivation centers of Taiwan. Providing necessary moisture content in the mushroom cultivation unit and the presence of dead plant materials influences the growth of slime moulds.

NUTRIENT CONTENT ANALYSIS

Oyster mushroom has a good nutritious value and is made of an essential source of proteins, carbohydrates, minerals, calcium and iron **(Hilal *et al.*, 2012)**. It has been used as an important food since centuries. Mushroom consumption is growing day by day due to its beneficial role in human wellbeing, therapeutic properties and nutrition **(Mshandete, 2011)**. *Pleurotus* species are attributed to some medicinal properties including anticancer, anti-inflammatory, antiviral activities, antibiotic, immune- modulator effect and blood lipid lowering effects and are effective against certain life threatening diseases **(Lavi *et al.*, 2010).** Its extract can effectively lower the cholesterol level **(Khatun *et al.*, 2007).**

The actual protein content depends on the composition of the substrate, size of the pileus, harvest time and mushroom species (**Bernaś *et al.*, 2006).** Mushrooms have been used in health care to treat simple and age-old common diseases like skin diseases and also pandemic diseases (**Garcia-Lafuente *et al.*, 2011**).

Oyetayo and Ariyo (2013) and **Dipan *et al.* (2018)** reported that *Pleurotus ostreatus* is one of the most known functional food for human health and its high nutritional values made it suitable for medicinal usage. According to **Finimundy *et al.* (2013)** and **Adebayo and Oloke (2017)** and mushrooms are cheap and nutritionally valuable victual which has anticancer, antioxidant, immune modulating, cardiovascular protector, anti-cholesterolemic, antiviral, antibacterial,

antidiabetic, anti-allergic, anti-parasitic, antifungal, detoxification and hepato protective effects.

QUALITATIVE ANALYSIS

Edible mushrooms act as valuable source of bioactive compounds **(Rajewska and Balasinska, 2004)**. The beneficial medicinal effects of mushroom improve resistance against various diseases and metabolic disruptions. The usage of edible mushroom extracts tends to be safer and decreases the possibility of undesirable side effects **(Poucheret, 2006)**. Mushroom contains a variety of secondary metabolites including phenolic compounds, steroids, and terpenes etc. **(Turkoglu *et al*., 2007).**

Phytochemical assay of 12 species of mushroom belong to five genera - *Agaricus* spp. (four species), *Coprinus* spp. (two species), *Panaeolus* spp. (two species), *Psathyrella* spp. (three species) and *Scleroderma* sp., showed the dominant presence of polysaccharides, saponins and tannins (**Maktoof *et al.*, 2019**). **Edet *et al.* (2016)** documented the existence of secondary metabolites such as alkaloid, glycosides, saponin, polyphenol, flavonoid, tannin, reducing compound and absence of phlobatannin, anthraquinone and hydroxymethyl anthraquninone in those species. Oyster mushrooms have been identified as a good source of phenolics, alkaloids and flavonoids (**Ganeshpurkar *et al.*, 2012).**

Aqueous extract of *Pleurotus florida* was reported to have the presence of alkaloids, flavonoids, terpenoids, steroids and cardiac glycosides, whereas its ethanolic extract showed the presence of alkaloids, flavonoids, terpenoids, saponins and steroids. Certain phytochemical compounds such as phenols, tannins, pholobatannins and anthroquinones were absent in both the extracts

(Manimaran *et al.*, 2017). Prabu and Kumuthakalavalli (2017) examined the methanolic extract of *Pleurotus florida* which confirm the presence of some phytochemicals namely phenols, flavonoids, saponins, tannins as well as terpenoids.

QUANTITATIVE ANALYSIS

Khan *et al.* (2009) investigated the total protein, carbohydrate, lipid, crude fiber and mineral (ash) contents of *Agaricus bisporous, Pleurotus citrinopleatus, Pleurotus eryngii, Auricularia polytricha, Ganoderma lucidum, Hypsizygous ulmarius, Agrocybe aegerita, Volveriella volvacea, Lentinus edodes, Coprinus comatus* and *Coriolus versicolor.* The protein, carbohydrate, total lipid, crude fiber, and total mineral contents ranged from 18g to 38g, 9g to 50g; 1g to 12g; 8g to 52g and 5g to 12g per 100g of the mushroom species respectively. The metabolizable energy content of these selected mushrooms ranged from 150 Kcal /100 kg to 300 Kcal /100 kg of mushroom.

Mshandete and Cuff (2007) analyzed the proximate content of three wild edible mushrooms namely *Coprinus cinereus, Pleurotus flabellatus, Volvariella volvaceae* using dried sample. Amount of protein, vitamin C, minerals, crude fiber, carbohydrate, fat content and energy values ranged from 17-28 %, 33-55 mg/100 g, 5.2-3232 mg/100 g, 6.6-11 %, 50-62 %, 1-3.3%, 302-313 kcal/100 g respectively. Research conducted by **Banik and Nandi (2004)** informed that supplementation of rice straw with biogas residual slurry manure has significant effect on the improvement of the yield capacity, protein and mineral nutrient contents of *Pleurotus sajorcaju* mushroom.

Proximate composition and mineral content of five species of edible mushrooms namely *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus florida,*

Pleurotus HK- and *Calocybe indica* were examined. Protein, lipids, carbohydrates, dietary fiber and total carbohydrates content in mushrooms were present between the range of 3.22 g to 4.83 g, 0.41 g to 1.05 g, 4.2 g to 6.37 g, 0.58 g to 1.11 g and 4.82 g to 7.48 g per 100 g of fresh edible portion respectively. Moisture, total solids, ash content and total calorie value was found to be in the range of 85.95 g to 90.07 g /100 g, 9.93 g to 14.05g/100 g, 0.98g to 2.3g/100 g and 35.51g to 50.03 Kcal / 100 g of fresh weight of the edible portion respectively. The values of Zinc, copper, iron, sodium, potassium, calcium, magnesium, phosphorus and manganese content in mushrooms were found to be in the range of 0.65-1.24, 0.14-0.91, 0.94- 1.81, 3.18-37.23, 19.83-197.24, 0.12-0.58, 27.26-51.21, 22.2-62.1 and 0.17-0.53 mg/100 g of fresh weight respectively **(Zahid *et al.*, 2009)**.

Akindahunsia and Oyetayob (2006) studied the nutrition composition of different parts of *Pleurotus tuber-regium*. In the macronutrient composition (g/100 g), crude protein varied from 4.1 g to 13.8 g, with the highest in the cap (13.8 g) than any other parts and total carbohydrates content ranged from 34.0 g to 56.2 g, while the crude fat and ash contents were low. Potassium was found to be the highest concentration (mg/g) in the stalk (3.3 g) while copper was present in trace amounts only. Research of **Alam *et al.* (2008)** suggested that dietary mushrooms namely *Pleurotus ostreatus, Pleurotus sajorcaju, Pleurotus florida and Calocybe indica* were rich in essential nutrition's and it had 20 - 25% of protein, 13 - 24% of fibers,4 - 5% of lipid, 37 - 48% of carbohydrate, 8 - 13% of mineral content and 86 to 87.5% of moisture content.

Sardar *et al.* (2017) reported that higher proteins, carbohydrates, fats, macro (P and K) and micronutrients (Zn, Fe, Na, Mg, Mn and Ca) were observed in

Pleurotus eryngii cultivated on cotton waste when compared to wheat straw, rice straw, corn cobs, sugarcane bagasse and sawdust. **Khan (2010)** reported the nutrient content of *Pleurotus* species which consisted 17 to 42 g of protein, 37 to 48 g of carbohydrates, 0.5 to 5 g of lipids, 24 to 31 g of fibers, 4 to 10 g of minerals and 85 to 87 % of moisture content. **Tirkey *et al.* (2017)** reported the variation in the quantity of the nutrient content of *Pleurotus florida* cultivated upon different substrate. They observed the highest moisture content (92%), protein (32.20%) carbohydrate (4.46%) in grass, cardboard + banana leaves, wheat straw respectively. Lipid content was found to be higher (0.88%) in wheat straw and cardboard + banana leaves combination followed by other substrates.

Salami *et al.* (2017) reported 26.28 – 29.91% protein, 86.90 – 89.60% moisture, 0.48 – 0.91% fat, 19.64 – 22.82% fiber, 31.37 – 38.17% carbohydrate and 5.18-6.39% ash in *Pleurotus florida*. The mineral composition varied from 0.3 – 0.4 g/100 g of calcium, 1.0 – 1.1 g/100 g of phosphorus, 0.017 – 0.021 g/100 g of Iron, 0.3 – 0.4 g/100 g of sodium and 2.1 – 2.3 g/100 g of potassium. Different substrate such as cotton waste, *Cymbopogon citrates* leaves and *Panicum maximum* leaves were used for the cultivation of *Pleurotus pulmonarius* by **Silva *et al.* (2002)**. The results showed higher protein (29.19%) and fiber (9.0%) for the mushroom which was cultivated on cotton peel.

Pleurotus species contain some essential fatty acids. Oleic acid is the major monounsaturated fatty acid and linoleic acid is the major polyunsaturated fatty acid found in *Pleurotus ostreatus*. According to the study of **Hossain *et al.* (2007)**, *Pleurotus ostreatus* was made up of (monounsaturated fatty acids) oleic acid (363 µg/g dried mushroom) and the (n-6 essential fatty acids) linoleic acid

(533 µg/g dried mushroom). Linoleic acid (11.6 µg/g dried mushroom) and arachidonic acid (10.8 µg/g dried mushroom) were also observed in *Pleurotus ostreatus*. As a whole, the lipid concentration of *Pleurotus* species ranged from 0.2 to 8g per 100 g of dried fruiting bodies was recorded from various experiments with different species **(Hossain *et al.*, 2007).**

Devi and Krishnakumari (2015) studied the chemical composition of *Pleurotus sajor-caju* using aqueous extract which showed 2.53 ± 0.40 mg/g of carbohydrate, 7.59 ± 0.23 mg/g of protein and 2.89 ± 0.30 mg/g of amino acids.

ANTIOXIDANT CONTENT AND INVITRO ANTIOXIDANT ACTIVITY

There is a common statement that "medicines and foods have a common origin" **(Kaul, 2001)**. Antioxidant capacity of mushrooms can suppress the active oxygen species, which are related to aging and diseases **(Yang *et al.*, 2002).** Some antioxidants like vitamin A and flavonoids are able to prevent different types of sickness **(Geosel *et al.*, 2010).** Presence of polyphenol compound is related to the antioxidant properties. Consumption of polyphenol-rich foods and beverages can reduce the risk of stroke, cardiovascular diseases and certain types of cancer **(Barros *et al.*, 2007; Jagadish *et al.*, 2009).** Consumption of dietary antioxidants can prevent the free radical damage. According to **Olajire and Azeez (2011)**, antioxidants have the ability to scavenge free radicals by inhibiting the initiation step or interrupting the spread of oxidation of lipid and delaying of oxidation by several actions.

Pleurotus species serves as a valuable source of variety of antioxidant compounds namely flavonoids, phenolic compounds, carotenoids, vitamins C and vitamin E **(Jayakumar *et al.*, 2011)**. Methanolic extract of *Pleurotus florida* showed

efficient activity against free radicals (**Menaga *et al.*, 2013**). Presence of amino acids, nucleotide and some elements such as nitrogen, phosphorus, potassium, sulphur, iron, zinc and autoxidation of unsaturated fatty acids are responsible for the aroma **(Bernas *et al.*, 2006)**.

The investigations of *Armillaria mellea*, *Boletus badius*, *Boletus edulis*, *Cantharellus cibarius*, *Lactarius deliciosus* and *Pleurotus ostreatus* revealed the presence of the following acids: protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic, sinapic, vanillic and cinnamic. Composition and the volume of the nutrient content in these species is diverse. The phenolic content ranged from 6 mg/kg DW in *Armillaria mellea* to 48.25 mg/kg DW in *Boletus badius*. A Protocatechuic acid amount ranged between 1.37–21.38 mg/ kg DW, with its maximum in *Boletus badius*. p-Hydroxybenzoic and sinapic acid predominate in *Pleurotus ostreatus*. Cinnamic acid was found to be higher in *Boletus badius*. Thus, the results prove that edible mushrooms are a valuable dietary source of phenolic acids with antioxidant activity (**Muszyska *et al.*, 2013).**

Among all the commercial mushrooms, fruiting bodies of *Pleurotus* species had higher concentration of antioxidants (**Mau *et al,* 2001; Yan *et al.,* 2002**). A polysaccharide compound pleuran (β-glucan) was isolated from *Pleurotus ostreatus* and expressed a positive effect on rat colon with pre-cancerous lesions (**Bobek and Galbavy, 2001**). *Pleurotus ostreatus* can strengthen certain antioxidant enzyme such as superoxide dismutase, catalse and peroxidase thereby reducing the oxidative damage in human (**Yan *et al.,* 2002; Keyhani, 2007).**

Abdullah (2012) studied the phenoic content and antioxidant capacities of selected culinary-medicinal mushrooms namely *Agrocybe* sp.,

Auricularia auricular-judae, Flammulina velutipes, Ganoderma lucidum, Hericium erinaceus, Lentinula edodes, Pleurotus cystidiosus, Pleurotus flabellatus, Pleurotus sajor-caju, Pleurotus eryngii, Pleurotus florida, Termitomyces heimii, Schizophyllum commune, and *Volvariella volvaceae*. Phenolic content was stated to be higher in *Ganoderma lucidum* and the values ranged from 6.19 - 63.51 mg GAE/g. *Flammulina velutipes* had the lowest overall total antioxidant index and the maximum amount was observed in *Ganoderma lucidum.* **Devi and Krishnakumari (2015)** reported 2.81 ± 0.61 mg/g of alkaloid, 5.36 ± 0.31 mg/g of flavonoid, 3.35 ± 0.20 mg/g of phenol, 6.84 ± 0.12 mg/g of tannin, 0.87 ± 0.03 mg/g of vitamin A, 1.13 ± 0.03 mg/g of vitamin C and 0.52 ± 0.01 mg/g of vitamin E in the aqueous hot extract of *Pleurotus sajor-caju*. **Gan *et al.* (2013)** estimated the overall phenolic and flavonoid content of *Agaricus bisporous* (white button mushroom) and *Agaricus brasiliensis.* Total phenolic and flavonoid concentration ranged between 12.50 - 21.47 mg/g DW and 1.36 – 5.36 mg/g DW respectively.

Mushroom fruiting bodies are abundant in vitamins, primarily constitute of vitamin B_1, vitamin B_2, vitamin C and vitamin D_2 (**Manzi *et al.*, 2004**). In vitamin B group, particularly thamine, riboflavin, pyridoxine, pantotene acid, nicotinic acid, nicotinamid, folic acid and cobalamin as well as other vitamins such as ergosterol, biotin, phytochinon and tocopherols are abundant **(Mattiala *et al.*, 2001).**

Pumtes *et al*. (**2016**) analyzed the antioxidant activities in terms of total phenol, flavonoid content, ABTS and DPPH assays by different extraction method and solvent concentration. *Pleurotus flabellatus* showed 215.26–301.76 mg GAE/100 g and 14.57 - 31.17 mg QCE/100g of phenols and flavonoids respectively. Highest IC_{50} (mg/ml) value obtained from DPPH and ABTS

radical scavenging activity assay were 3.15 and 13.33 respectively. **Shoba and Krishnakumari (2018)** reported antioxidant IC_{50} for the *Pleurotus eous* methanol and aqueous extract. Hydroxyl radical scavenging activity was found to be 290, 440 µg/ml and Hydrogen peroxide scavenging activity was 475, 370µg/ml and antidiabetic (α Amylase) IC_{50} was found to be 460, 500 µg/ml and (α Glucosidase) 325, 280 µg/ml respectively.

Ebrahimzadeh *et al.*, (2010) evaluated the antioxidant activity of ethyl acetate and methanol extracts of *Cantharellus cibarius* and *Pleurotus porrigens* by using six in vitro assays. Ethyl acetate extracts exhibited more amounts of flavonoids and phenolic contents than methanolic extract. Higher DPPH scavenging activity and Fe^{2+} chelating activity was found to be higher in methanolic extract than ethyl acetate extract.

Sudha *et al.* (2012) have studied the antioxidant potential of ethyl acetate, methanol, and hot water extracts by utilizing *Pleurotus eous* using various *in vitro* Reactive Oxygen Species / Reactive Nitrogen Species generate chemical and biological models. Their findings showed that *Pleurotus eous* served as the natural antioxidants owing to their significant antioxidant activity. It had the capacity to scavenge the DPPH, ABTS and hydroxyl radicals. It also possessed metal chelating ability, reducing capacity in Fe^{3+}/ferricyanide complex, ferric reducing antioxidant power, and inhibit lipid peroxidation.

Ragupathi *et al.* (2018a) investigated the antioxidant activities of crude methanol extracts of ten wild mushrooms namely *Trametes versicolor, Calvatia gigantean, Gymnopilus junonius, Cortinarius* sp., *Tricholoma* sp., *Mycena* sp., *Coprinus comatus, Amanita muscaria* and *Tricholoma equestre* collected from

Southern Western Ghats. Of the ten species analyzed, *Gymnopilus junonius, Tricholoma equestre and Trametes versicolor* displayed higher antioxidant activity both in nitric oxide scavenging assay and DPPH scavenging assay. **Alispahić *et al.* (2015)** observed various levels of polyphenolic content and anthocyanins content as well as radical scavenging activity of five edible mushrooms namely *Boletus edulis, Agaricus bisporus, Agaricus bisporus var. avellaneous, Pleurotus ostreatus, Lentinula edodes*.

Sumathy *et al.* (2013) reported high percentage of inhibition for methanol and moderate inhibition for chloroform extracts of *Pleurotus citrinopileatus* by DPPH radical scavenging assay. Similarly, **Keleş *et al.* (2011)** reported significant antioxidant activity against various *in vitro* antioxidant systems using methanolic extract of 24 wild edible mushroom species.

Sharma and Gautam (2015) studied 20 wild culinary mushroom species which is taken as food by the people living around Northern Himalayan regions. They reported the bioactive and antioxidant potential of the selected edible mushrooms. Among twenty mushrooms, *Gymnopilus junonius* was found to have higher antioxidant activity. Presence of diverse antioxidant molecules in several species of mushroom makes it an attractive food **(Guerra-Dore *et al.*, 2007).** Presence of flavonoids in mushroom serves as a free radical scavenger which can terminate the radical chain during triglycerides oxidation (**Barros *et al.*, 2008).**

Methanol extracts from *Pleurotus ostreatus* exhibited antioxidant activity, reducing power, radical scavenging and iron chelating activities, which were significantly higher than the other commercial mushrooms **(Yang *et al.*, 2002).** On the other hand, some workers reported that oyster mushroom extracts possessed only

moderate antioxidant activities compared to the other edible mushrooms **(Elmastas *et al.*, 2007 and Dubost *et al.*, 2007)**.

Primary factor in the progression of many degenerative diseases is oxidative stress. Some antioxidant compounds like phenols and flavonoids are delaying and inhibiting the compounds responsible for causing oxidative stresses **(Adebayo *et al.*, 2014; Okafor *et al.*, 2017)**. **Jayakumar *et al.* (2011)** reported that *Pleurotus ostreatus* extract improves the catalase gene expression and decrease the occurrence of free radical-induced protein oxidation in older rats, which decreases the age related disorders. **Hapsari *et al.* (2012)** and **Okafor *et al.* (2017)** report *in-vitro* and *in-vivo* antioxidant activities of ethanolic extract of the oyster mushroom which showed potential free radical scavenging activity. Polysaccharide compounds which were isolated from *Pleurotus ostreatus,* PSPO-1a and PSPO-4a, exhibited stronger DPPH and superoxide anion radical scavenging activity with increase concentration. However, they were less effective against hydroxyl radical. Of the two polysaccharides tested, PSPO-1a was more successful against free-radicals than PSPO-4a **(Zhang *et al.*, 2012)**. Later, **Mitra *et al.* (2013)** reported that the free radical scavenging properties of the water-soluble polysaccharides from *Pleurotus ostreatus* had superior antioxidant properties, which might be due to the presence of β-glucan.

FTIR

FTIR spectroscopy is one of the possible ways to identify the biomolecule present in a mushroom sample (**Philip, 2009**). Infrared spectrum helps to study the polar bonds present between the atoms of sample molecules and vibrations of molecule. The mushroom extract spectrum showed the presence of OH groups,

CH_2 asymmetric stretch/ symmetric stretch, secondary amines, sugars, glycosides and C-H groups (**Bains and Tripathi, 2016).**

Nojehdehi *et al.* (2016) observed ester linkages, carbonyl, NH, NH_2, amide I (which is created because of the vibrations of carbonyl stretch bonded to the protein), C=N bond, primary amines and OH groups (which were related to the proteins) in *Agaricus bisporus*. **Sanjana *et al.*, (2013)** focused on the water-soluble glucan from the *Pleurotus ostreatus* extract. It primarily composed of glucose, mannose and fucose in a ratio of 3:2:1 with both β and α linkage. A heteroglycan was isolated from the mycelia of *Pleurotus ostreatus.* The later it is found to be consisted of L fucose, D-mannose, and D-glucose in a molar ratio of around1:2:3 **(Patra *et al.*, 2013).**

Narasimha *et al.* (2011) reported the presence of main functional groups of *Agaricus bisporus* namely proteins, carbohydrates, dietary fibers, carbonyl groups, hydroxyl and carboxylic acids. **Lamrood and Raleganker (2017)** reported the FTIR results of water-soluble polysaccharides from *Phellinus merrillii*. Spectra confirmed the presence of polysaccharides and proteins β-D-glucan, β-D-Galactose in the extract. **Chen *et al.* (2014)** reported that peaks at 1661 and 1629 $cm^{-1}$is combined with peak at 1565 cm^{-1} indicates secondary amide group (-CO-NH-C) due to presence of large amount of chitin and was consistent for glucan-chitin complex in the fruiting body of *Pleurotus tuber-regium.*

Presence of amide I at 1640cm^{-1} and 1652 $cm^{-1}$and amide II (due to bending vibrations of N-H group) at 1530cm^{-1}(**Radzki and Kalbarczyk, 2010; Moharram *et al.*, 2008)** were reported in edible mushrooms *Lentinula edodes*, *Pleurotus ostreatus* and *Agaricus blazei*. **Das *et al.* (2020)** compared the IR spectra of *Pleurotus ostreatus, Hypsizygus ulmarius, Pleurotus sajor-caju,*

Pleurotus cornonucopiae, Pleurotus platypus and *Pleurotus flabellatus.* Spectra displayed the presence of glucan band at 890 cm^{-1} it particularly indicates the presence of β-1,3-glucan, while 806 cm^{-1} and 952 cm^{-1} were confirmatory bands of mannan type glucan and chitin. Cell membrane phospholipid was confirmed by amide I band at 1747 cm^{-1}.

GCMS

The bioactive components of *Pleurotus ostreatus* had been evaluated using GC/MS by **Priya *et al.* (2012).** GC/MS study of hydroalcholic extract of *Pleurotus ostreatus* indicated the existence of Cholestane-3,7,1,25-tetrol tetraacetate, (3a,5a,7a,12 a)-55.20, 9,12-Octadecadienoic acid, methyl ester(E,E)-18.55,14,17-Octadecadienoic acid, methyl ester(E,E)-5.59,Pentadecanoic acid, ethyl ester-3.84. The results confirmed that *Pleurotus ostreatus* can be used as alternative for the current synthetic antimicrobial agents.

Johnathan *et al.* (2016) reported several active compounds in *Lignosus rhinoceros* such as fatty acid group 68.58% followed by fatty acid esters 10.18%, sterols 6.26%, amides 5.76%, carboxylic acids 3.01%, alcohols 1.64%, alkanes 1.26% and ketones 1.3%. The most frequent component was linoleic acid 49.39%, followed by palmitic acid 11.29% and linolelaidic acid methyl ester 7.4%.

Alshammaa (2017) identified the presence of 39 components such as Glycerin, Hexane, 2,4,4-trimethyl-, 2-Pyrrolidinone, 1,2,4,5-Tetrazine, hexahydro-1,2,4,5-tetramethyl, 4-Pyridinecarboxylic acid, 2-Furanmethanamine, tetrahydro-N-[(tetrahydro-2-furanyl)methyl], DL-Proline, 5-Oxo-, methyl ester, Niacinamide, DL-Phenylalanine, methyl ester, 2-Pyrrolidinecarboxylic acid-5-Oxo-, ethyl ester, S-[1-Phenyl-2-[2,2-dimethylpropyl] aminoethyl] thiosulfate,

2-Cyclohexen-1-one, 2-hydroxy-4,4,6,6-tetramethyl, 1H-Pyrrole, 1-(4-methylphenyl), 2-Acetyl- 1,5-dimethyl-8-oxabicyclo [3.2.1] octane, Pentadecanoic acid, methyl ester, Octadecanoic acid, methyl ester, Anthiaergosatn-5, 7,9,22-tetraen, 3-acetoxy etc., by GC-MS analysis in *Agaricus bisporus* and *Pleurotus ostreatus.*

Scientific research of previous authors reported the presence of certain vitamin B complex members such as niacin, flavin and pyridoxine, organic acids namely glucons, monoterpinoids and diterpinoides, lipids, proteins like a hydrophobins and trace element such as selenium in *Lentinus edode*, *Grifola froundosa*, *Agaricus bisporus* and oyster mushroom (**Kinger and Fahey, 2005; Valentao *et al.*, 2005).**

Petrova *et al.* (2007) studied the volatile fractions and butanol extracts results in the identification of 44 compounds from *Agaricus placomyces* and 34 compounds form *Agaricus pseudopratensis.* Phenol and urea was the most abundant constituent in the volatiles and butanol extract. They also report the presence of ergosterol and two Δ7-sterols. For the first time 5α, 8α-Epidioxi-24(ξ)-methylcholesta-6, 22- diene-3β-ol is isolated from *A. placomyces* and *A. pseudopratensis*.

Termitomyces heimiis is a wild edible mushroom commonly used as food by the Trible communities in Agency area of Visakhapatnam. In the study **Santhikumari *et al.* (2019)**, confirmed the existence of the volatile compounds such as 4-hydroxy-3-methoxybenzyl alcohol (0.008%), Flavone (0.035%), Estra-1,3,5(10) trien–trien-17a-ol (0.241%), Phytol (0.107%), Octadec – 9 - enoic acid (0.483%), 2,6 - Bis (1,1-dimethylethyl) – 4 – phenylmethylenecyclohexa - 2, 5-din-1-one (0.025%), Octadecanoic acid,3-oxo- methyl ester(0.024%), 1H – Pyrrol (2,3-b) quinoxalin-2-imine, 2,3,3a,4,9,9ahexahydro-1,N-diphenyl - (0.048%), Benzoic acid,2,4-dimethoxy-

6-methyl-, (8,8-dimethoxy-2octyl) ester (0.010%), 1,2-Proopanediol, bis(N phenylcarbamate) (0.014%).

Ragupathi *et al.* (2018b) also noticed several prevalent compounds from methanolic extract of *Tricholoma equestre* namely 9,12-Octadecadienoic acid (Z,Z), Ergosta-5,8,22-trien-3-ol, (3.beta., 22E), Octadecanoic acid, 2,3-dihydroxypropyl ester, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z) -, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Octadecanoic acid, 1-Dodecanol, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, n-Pentadecanol and Dodecane.

Suseem and Saral (2013) performed the GM-MS analysis of petroleum ether extract of dried fruiting bodies of *Pleurotus eous*. They identified fatty acids esters such as Cyclopentanetridecanoic acid, methyl ester (25%), Tartronic acid, (p-ethoxyphenyl), diethyl ester (16.3%), 7, 10-Octadecadenoic acid, methyl ester (40.3%), Heptadecanoic acid, 16-methyl, methyl ester (13.5%) and 9-Octadecenoic acid [Z]-, 2-hydroxyl-1-[hydroxymethyl] ethyl ester (4.9%).

Ramos *et al.* (2018) experiment revealed the presence of major and minor compounds of *Pleurotus ostreatus* indicates their medicinal potential which may enhance the health and wellness of human life. GC-MS examination of the methanol extract of *Pleurotus ostreatus* confirmed the presence of thirty major compounds along with glycerin (23.36%), formamide (10.62%), 2-nonanol (9.92%), cyclobutanone, 2- methyl-4-hydroxy-(9.67%) and 1,1,2-trimethyl-3,8,9-trioxa-bicyclo[4] (5.62%) and 25 minor constituents.

Falade *et al.* (2017) reported that *Rigidoporus microporus* composes of active compounds such as hexadecane, n-hexadecanoic acid, pentadecanoic acid,

octadecane, hexadecanoic acid, n-octadecenoic acid, di-n-octyl phthalate (1,2benzenedicarboxylic acid, dioctyl ester) and n-decanoic acid.

ANTIMICROBIAL ACTIVITY

Continuous use of synthetic antibiotics made the pathogen to become resistance which leads to clinical difficulty while treating them (**Tambekar *et al.*, 2006**). Interest of reinvestigation of medicinal mushrooms and some other members of Basidiomycetes have been increased as a result of increasing difficulty and the cost of isolating novel bioactive compounds from the Actinomycetes and Streptomycetes. Growth of chosen bacteria species such as *Staphylococcus aureus* and *Bacillus cereus* were inhibited by five mushrooms out of six selected. In addition to this, there was a marked increase in their antimicrobial activity when the synthetic antimicrobial discs coupled with mushroom extract (**Karwa and Rai, 2009**).

Pleurotus ostreatus defenses against various microorganisms. The extract and its isolated compounds can be used as antibacterial and antifungal agents. Organic acid extract from *Pleurotus ostreatus* contain p-anisaldehydewhich has inhibitory effects on *Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger* and *Fusarium oxysporum* **(Okamoto *et al.*, 2002; Periasamy, 2005; Okafor *et al.*, 2017).**

The antimicrobial potency of *Pleurotus ostreatus* petroleum ether and acetone extracts were observed to inhibit the growth of some gram positive and gram negative bacteria **(Iwalokun *et al.*, 2007)**, whereas **Karaman *et al.* (2010)** reported that methanol and chloroform extract of *Pleurotus ostreatus* were effective against Gram-positive bacteria. **Mirunalini *et al.* (2012)** also studied the antibacterial activity

of *Pleurotus ostreatus* extract and biosynthesized silver nanoparticales in which synthesized silver nanoparticles showed maximum zone of inhibition.

Sivakumar *et al.* (2006) reported the antibacterial activity of *Osmoporus odoratus* using different solvent extracts against *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa*. Among various extracts used, water extract showed maximum antibacterial activity against the tested organisms.

Ramesh and Pattar (2010) determined the antimicrobial activity of *Ramaria formosa, Lycoperdon perlatum, Clavaria vermiculris, Maramius oreades, Cantharellus cibarius* and *Pleurotus pulmunarius* against selected group of pathogenic bacteria and fungi. Concentration of bioactive compounds present in the mushroom directly influences their performance in inhibiting microbial growth of these selected strains.

Quershi *et al*. (2010) studied the antimicrobial efficiency of specific solvent extracts of *Ganoderma lucidum* (40µg/ml) towards six bacterial pathogens. Maximum antibacterial activity (31.60 ± 0.10) was observed in acetone extract. Effect of ethanollic extracts of *Pleurotus sajor-caju, Pleurotus florida* and *Pleurotus aureovillosus* are tested against five Gram-negative bacteria, four Gram-positive bacteria, and one yeast species. Performance of *Pleurotus* species showed very narrow antibacterial spectrums against Gram-negative bacteria but the activity was found to be higher against Gram-positive bacteria **(Loganathan *et al*., 2008)**.

Sheena *et al.* (2003) reported the antibacterial activity of selected macro fungi *Ganoderma lucidum, Navesporus floccose* and *Phellinus rimosus* that are found in

South India, and were found to be effective against selected bacterial strains. Newly identified sesquiterpenoid hydroquinones isolated from European *Ganoderma pfeifferi* performed well against selected bacterial species including methicillin- resistant *Staphylococcus aureus* and some other bacterial species **(Mothana *et al.*, 2000)**. **Badalyan (2004)** reported that the presence of oxalic acid present in the *Lentinula edodes*is responsible for its inhibiting activity against several bacterial including *Staphylococcus aureus*.

Schillaci *et al.* (2013) reported the varying degree of inhibitory activity of Mediterranean culinary-medicinal oyster mushrooms *Pleurotus eryngii* var. *eryngii*, *Pleurotus eryngii* var. *ferulae*, *Pleurotus eryngii* var. *elaeoselini* and *Pleurotus nebrodensis* against a group of bacterial reference strains such as *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus epidermidis* RP62A and *Escherichia coli* ATCC10536. **Pauliuc and Dorica (2013)** examined the antimicrobial activity of gemmotherapic extract from young parts of *Pleurotus ostreatus* against three Gram-positive bacteria (*Bacillus subtilis, Bacillus cereus* var. *mycoides* and *Streptococcus faecalis*) and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Serratia marcescens*) by using the well diffusion method. The extract had a significant inhibitory activity against *Bacillus subtilis* and *Bacillus cereus* var. *mycoides* but moderate on the other species and minimal on *Serratia marcescens*.

Tambekar *et al.* (2006) reported that the intake of mushroom can help in inhibiting the growth of common pathogenic bacteria. Different solvent extracts of *Agaricus bisporus* and *Pleurotus sajor-caju* showed considerable inhibitory activity against *Escherichia coli* 390, *Escherichia coli* 739, *Enterobacter aerogenes,*

Pseudomonas aeruginosa and Klebsiella pneumonia. Petroleum ether, methanol, ethyl acetate and aqueous extracts of *Pleurotus eous* were screened for its antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae.* Petroleum ether extract showed the highest antibacterial activity among all the crude extracts tested **(Suseem and Saral, 2013).** *Pleurotus ostreatus* and *Lentinula edodes* showed less antibacterial activities with less than 10 mm of inhibitory zones against gram negative (*Escherichia coli* and *Pseudomonas fluorescens*) and gram positive bacteria (*Bacillus cereus, Stapylococcus aureus* and *Ganoderma lucidum*) display higher inhibition zone varied from 11 mm to 16 mm **(Islam *et al.*, 2015)**.

SYNTHESIS SILVER NANO PARTICLES

In current scenario much interest on biological mediated synthesis of nanoparticles using plants, fungi, microbes and yeast increasing day by day **(Philip, 2009; Tripathy *et al.,* 2009).** Extracts taken from bio-organisms may have capacity to act both as reducing and capping agents in silver nanoparticles synthesis. Ag^{+} ions interact with the biomolecules present in these extracts, such as enzymes/proteins, amino acids, polysaccharides and vitamins. These processes are achieved either through intracellular or extracellular synthesis **(Acosta, 2009)**.

Silver nanoparticles combined with active compounds have been extensively used as medicine for treatment and to induce wound healing activity **(Vermeulen, 2010)**. Silver nanoparticles possess high specific area than their volume, which will have a good antimicrobial activity as compared with bulk Ag metal **(Mahendra *et al.,* 2009)**. Antimicrobial activities of synthesized silver nanoparticles are well demonstrated against both bacteria **(Sondi and Salopek-Sondi, 2004)** and

viruses (**Lara, *et al.*, 2010**). This is due to the close attachment of the nanoparticles surface with microbial cells or viruses. This antimicrobial activity is also dependent on the size. Silver nanoparticles are currently playing an important active role in targeted drug delivery **(Jain *et al.*, 2009)** and artificial implants **(Sachlos, *et al.*, 2006)** also as a diagnostic agent for sensing and imaging of different diseases at their early stages. Although of its mutation-resistant antimicrobial properties, they are being used in various medical products such as antibacterial dressing **(Vigneshwaran, *et al.*, 2007)**, burn ointments **(Ip *et al.*, 2006)** and covering for medical devices **(Furno *et al.*, 2004)**. As studies on silver nanoparticles accelerates, its wide medical applications including infection prevention and wound healing applications also further widen **(Rujitanaroj *et al.*, 2008).**

Raman *et al.* (2015) performed an experiment in biosynthesis of silver nanoparticles using an aqueous extract of *Pleurotus djamor* var. roseus and its cytotoxicity against human prostate carcinoma (PC3) cells. Their finding indicated that silver nanoparticles may have antiproliferative effect on the PC3 cell line by restricting its growth, limiting DNA synthesis and inducing apoptosis.

Several workers attempted to utilize fungi as a source for synthesizing silver nanoparticles and gold nanoparticles (e.g., *Verticillium*, *Fusarium oxys-porum*, *Aspergillus fumigatus*, *Volvariella volvacea*, *Pleurotus florida*, *Ganoderma neo-japonicum*) **(Philip, 2009; Bhat *et al.*, 2011; Gurunathan *et al.*, 2013)**. Many biologically active compounds present in Basidiomycota have increased the interest towards this phylum **(Yashvant *et al.*, 2012).**

Bhat *et al.* (2011) performed an experiment on photo-irradiated extracellular synthesis of silver nanoparticles using the aqueous extract of culinary

oyster mushroom, *Pleurotus florida* as a reducing agent. The nanoparticles synthesized were also demonstrated impressive antimicrobial results, it can be recommended for using in several biomedical applications. Same activities were observed *Volvariella volvacea* and *Pleurotus sajor* **(Philip, 2009; Nithya and Ragunathan, 2009).**

Mirunalini *et al.* (2012) confirmed the development of silver nanoparticles using edible mushroom extracts. It confirmed that silver nanoparticles synthesized from edible mushroom extract will deserve to be a good candidate as an antibacterial agent. **Narasimha *et al.* (2011)** documented an eco-friendly protocol for the synthesis of sliver nanoparticles employing *Agaricus bisporus* (white button mushroom) extract. The presence of silver nanoparticles was confirmed by the presence of absorption peak at 420 nm. **Shivashankar *et al.* (2013),** demonstrated biosynthesis of silver nanoparticles and its antibacterial activity using some edible mushrooms like *Pleurotus pulmonarius, Pleurotus djamor* and *Hypsizygus (pleurotus) ulmarius*. Synthesized silver nanoparticles exhibiteded strong activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Abikoye *et al.* (2019) evaluated the biosynthesis of silver nanoparticles and anti-microbial properties of the UV mutant variety EW1 and EW1M1. Both of these mutant varieties of *Auricularia polytricha* exhibited higher antimicrobial activity than the wild type. Several workers have synthesized silver nanoparticles using *Pleurotus florida, Pleurotus sajorcaju, Pleurotus citrinopileatus, Pleurotus ostreatus, Pleurotus comucopiae var.* citrinopileatus, *Pleurotus salmoneostramineus* and *Pleurotus platypus* (**Philip, 2009; Balahanmuga *et al.*, 2013; Sujatha *et al.*, 2013;**

Bhat *et al.*, 2013; Sudhakar *et al.*, 2014; Owaida*et al.*, 2015; Maurya *et al.*, 2016; Owaid *et al.*, 2017)

UTILIZATION OF SPENT MUSHROOM SUBSTRATE AS A FERTILIZER

Spent mushroom substrate is an organic material left after harvesting mushroom fruit bodies. Production of edible mushroom on agro-industrial residues comprises more than 11 million tons of fresh mushrooms per year. Nearly 5 kg of spent mushroom substrate was obtained per kg of fresh mushroom production which leads to disposal problems. These organic wastes from mushroom industry, contains mycelium, lignin, cellulosic, hemicellulose, NPK (Nitogen-Phosphorous-Potassium) and metabolic liberates from mycelium (**Jasińska, 2018; Kadiri and Mustapha, 2010**). Research on spent mushroom substrate was focused commonly on the enzymatic activity of the residual mycelium and production of lignocellulosic enzymes namely laccase, xylanase, lignin peroxidase, cellulase and hemicellulase as a cheap source of bioremediation, animal feed and energy feedstock (**Santos *et al.*, 2005; Machado *et al.*, 2007; Azevedo *et al.*, 2009).**

SMS is most commonly used for compost preparation (**Ribas *et al.*, 2009; Medina *et al.*, 2009; Marques *et al.*, 2014**). During composting, the excess amount of mineral salts was washed out so that the decomposed substrate can be utilized as an organic fertilizer for the production of cereals, fruits, vegetables and ornamental plants cultivation (**Ahlawat and Sagar, 2007; Medina *et al.*, 2009; Hackett, 2015; Lopes *et al.*, 2015).**

Kadiri and Mustapha (2010) reported the effect of spent mushroom substrate on the vegetative growth and yield of cowpea and tomato. The composted spent

mushroom substrate added to loamy soil represents relatively high plant growth and yields of cowpea and tomato compare to the uncomposted spent mushroom substrate.

Jarecki *et al.* (2005) stated that spent mushroom compost leachates can be effectively recycled in plant culture as a potential source of nutrients. Tomato and marigold seedlings were treated with leachates from SMC and commercial compost expressed species dependent response. Tomato exhibited good growth in commercial compost leachate than SMC amendment leachate. In contrast, marigold showed excellent growth in SMC amendment leachate. Also, **Young *et al.* (2002)** applied SMC leachates to marigold, as a source of nutrients via fertigation, they recommend that SMC shall be used in amounts not exceed 50%. If the concentration exceeds, it affects the EC levels. Mixed application of air-dried *Pleurotus* SMS and *Agaricus* SMC in pepper plant cultivation revealed overall better performance in growth, protein, chlorophyll and carotenoids content **(Roy *et al.*, 2015).**

Zhang and Sun (2014) have composted green waste such as park, garden litter and trimmings, with spent mushroom substrate and biochar. From the study, it was concluded that green waste combined with 35% SMS and 20% biochar produced high quality compost in the shortest time of 24 days. Application of SMC at 40 and 80t ha^{-1} (moist) increases the yield of sweet corn, cabbage and potato. Also, authors suggested that timing for application of spent mushroom substrate to the soil should be approximately one month prior to the planting. This investigation also reveals the increase in respiration rate and phosphatase activity of the soil **(Medina *et al.*, 2012).**

Tajbakhsh *et al.*, (2008) have shown that vermicomposting process using SMS could be an alternative disposal strategy in an eco friendly way. Vermicomposted SMC was an alternative source of fertilizer which posses necessary

micro and macronutrients. It also supports the plant growth by providing good physical properties, low conductivity, low C: N ratio to the soil. Another researcher **Bakar *et al.* (2011)** demonstrated vermicomposting by employing red worms, *Lumbricus rubellus* of sewage sludge using *Pleurotus sajor-caju* SMS as a feed material.

Some valuable seedlings such as lettuce, tomatoes, peppers, cucumbers, tomatoes, broccoli, tulips, cauliflower, peppers, spinach, pumpkins, as well as soil mulching in orchards and strawberry plantations have been considered as an alternative substrate for production. The integration of SMS response varies from plant to plant **(Uzun, 2004; Ahlawat and Sagar, 2007 Medina *et al.*, 2009; Fidanza *et al.*, 2010; Zhang *et al.*, 2012**).

Lentinus subnudus is dried and supplemented with cultivation mixed with loamy soil for the propagation of cowpea and tomato seedlings, which give the best results as a growing medium. **(Kadiriand Mustapha, 2010)**. **Zhang *et al.* (2012)** assessed the effect of *Flammulina velutipes* SMS as a growth medium for the farming of cucumbers and tomato seedlings. Research reports have shown that *Flammulina Velutipes* SMS combined with perlite or vermiculite had sufficient physiochemical properties for the development of tomato or cucumber seedlings. **Segun *et al.* (2011)** have successfully cultivated *Lycopersicum esculentum, Abelmoschus esculentus, Capsicum annum* and *Capsicum chinense*on depleted garden soil supplemented with fresh SMS after *Pleurotus pulmonarius* cultivation.

Meiqin (2006) stated that the modification of the SMC complex organic fertilizer could maximize the amount of successful spikes and boost the ratio of mature spikes. In comparison with the control, SMC treatment increases spikes,

mature spikes which leads to the production of higher yield in rice. Properly sized and sieved fresh SMS mixed with vermiculture acts as an ideal growth medium for plants and provides exceptional aeration, porosity, water holding capacity and nitrogen. It acts as aconceivable substitute to peat in soilless mixer **(Romaine and Holcomb, 2001).**

Kwagyan and Odamtten (2018) investigated the influence of spent mushroom compost of *Pleurotus eous* strain P-31 on the growth and yield performance of pepper and tomato seedlings under green house conditions using different concentration in sandy loam soil. The results showed that Lower concentrations of SMC promoted plant height, leaf area, chlorophyll content, number of leaves and axillary branches of the two test plants.

MATERIALS AND METHODS

MATERIALS

Chemicals/Reagents	Company	Catalogue No.
Aluminium chloride	Nice chemicals	A15025 Batch No. 606610
Anthrone	Loba chemie	Batch No. G482808 CAS No. 90-44-8
Ascorbic acid	Rankem	Product code: A2842 Batch No. J206G10
Benedict's solution	Spectrum	Code: B0103 Batch No. 029906
Bovine serum albumin	Merck	CAS No: 90604-29-8 No. 11201800051730
Calcium carbonate	Rankem	Product code: C0057 Batch No. P15A100091 CAS No. 471-34-1
Chloroform	Isochem	C1001 BN: 534912
Cholesterol	Otto kemi	P. B. No.: 2850
DPPH	Himedia	GRM 791-5G Lot No: 0000224339 CAS No: 366-18-7
Ethanol	Jiangsu Huasi International trade Co. Ltd.	Batch No. 20150605
Ferric chloride	Spectrum	Lot No. 054312 Code: F0109
Folin-ciacalteau reagent	Spectrum	Lot No. 027411 Code: P0196
Galic acid	Himedia	GRM 233-500G Lot: 0000366982
Glacial acetic acid	Spectrum	Lot No. 71603 Code – A0101
Glucose	Himedia	GRM016-500G Lot – 0000156372
H_2O_2	Nice	Code – H10683 Batch No: 905074
Hydrochloric acid	Isochem	H1001 B. No. – 565001

Hexane	Spectrum	Lot No. 23606 Code H0109
Lead acetate	Spectrum	Code – L0106 Batch No. 266 203
Magnesium turnings	Loba chemie	UN. No. 1418 Lot S14101102
Ninhydrin reagent	Spectrum	Lot – 073708 Code No. 0108
Nitric acid	Spectrum	Code – N0102
n-propanol	Spectrum	Lot – 035606 Code – 10101
Nutrient agar	Himedia	GRM026-1000G CAS No. 9002-18-6 EC. No. 232-658-1
Nutrient broth	Himedia	M002-100G Lot No. 0000358212
Phenol	Spectrum	Lot – 069511 Code – P0106
Phosphoric acid	Nice	Code – 010129
Vanillin	Himedia	RM 616-100G Lot – 0000100117
Quercetin	Tci	Lot No. L6BBE-CJ EC No. 204-187-1
Silver nitrate	Isochem	S1075 B. No - 495001
Sodium carbonate	Spectrum	Lot – 332502 Code – 80107
Sodium hydroxide	Spectrum	Lot No. 269001 Code – S0116
Sodium nitrate	Himedia	RM-1184-500G Lot – 0000105011
Sodium potassium tartarate	Fischer	Code No. 10805 No. – 06405114
Sodium sulphate	Spectrum	Lot – 222603 Code – S 0178
Sulphuric acid	Isochem	B. No. 566001 S1001
Tannic acid	Isochem	T1015 B. No. 175907
Trichloro acetic acid	Himedia	RM6274-100G Lot – 0000139363
Wagner's reagent	Nice	B. No. 507255 B. Code – W25771

METHODS

COLLECTION OF AGRICULTURAL WASTE MATERIAL

Agro waste materials such as paddy straw, sugarcane bagasse and banana leaves were used for the preset study. Paddy straw and banana leaves were collected from farmers and sugarcane bagasse was purchased from sugarcane vendors. These substrates were dried and stored for further use.

PURCHASE OF SPAWN

Sorghum grain based mother spawn of *Pleurotus florida and Pleurotus ostreatus* were procured from certified cultivation centre, MSM Mushroom Corner, Mushroom Cultivation Training and Seed Sale, Rediyarpatti, Tirunenlveli and used for the preparation bed spawn for the present study.

PREPARATION OF BED SPAWN:

Bed spawn was prepared by following the standard procedure of Tamil Nadu Agricultural University. Spawn is nothing but the mushroom fungus grown on a grain based medium. Among several materials tested by TNAU, Coimbatore, sorghum grains are the best substrate for the excellent growth of the fungus. Disease-free sorghum grains are used as substrate for growing the spawn. Various steps involved in the preparation of mother spawn are listed.

(i) Grain Sterilization

Sorghum grains were soaked in clean water to remove chaffy and damaged grains. The soaked grains were cooked in a vessel for 30 minutes just to soften them. Avoid over cooking of sorghum grains, as over cooking lead to splitting of grains. The

cooked grains were taken out and spread evenly on the platform to remove excess water.

(ii) Mixing with $CaCO_3$

At 50% moisture level, the cooked grains were mixed thoroughly with calcium carbonate ($CaCO_3$) at 20g/Kg. The purpose of mixing calcium carbonate is to remove the excess moisture present in the cooked grains, to neutralize the pH of the grains and to avoid caking of grains after sterilization.

(iii) Filling

The grains were filled in saline bottles / polypropylene bags up to $3/4^{th}$ height (approximately 300-330 g / bottle), inserted a PVC ring, bold the edges of the bag down and plugged the mouth tightly with non-absorbent cotton wool. The cotton plug was covered with a piece of paper and was tied tightly around the neck with a jute thread.

(iv) Sterilization of Processed Grain

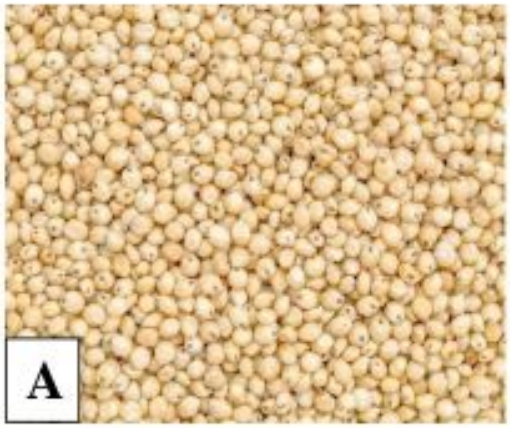
The bags were arranged inside an autoclave and sterilized under 20-lbs pressure for 2 hours. The bags were taken out after cooling and kept them inside the culture room under UV light for 20 minutes.

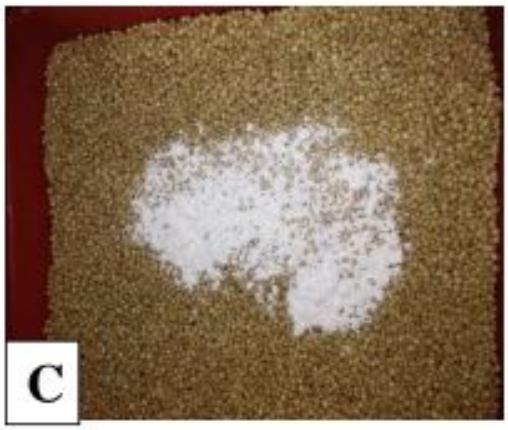
(v) Inoculation of Mother Spawn in Bed Spawn Media:

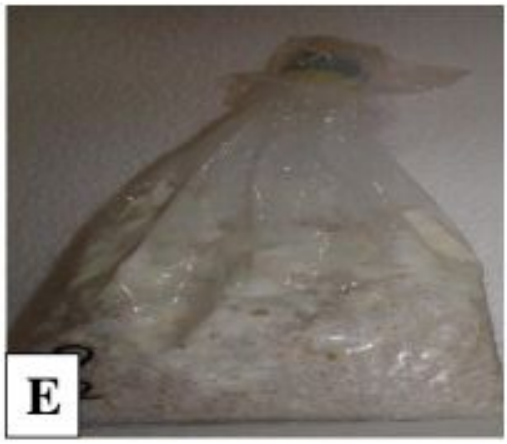
A small amount of mother spawn was aseptically transferred to bed spawn media. The bottles were incubated in a clean room under room temperature for 2 - 4 weeks. This was used as bed spawn.

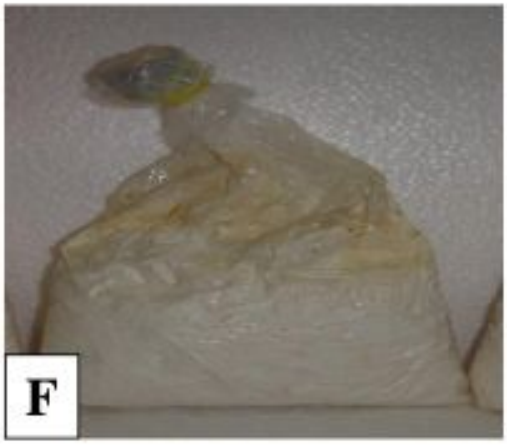
Plate 1

PREPARATION OF BED SPAWN









A – Sorghum grains

B – Boiled sorghum grains

C – Mixing with $CaCO_3$

D – Mycelium inoculated spawn bag

E – Partially colonized spawn bag

E – Fully colonized spawn bag (3^{rd} week)

CULTIVATION OF MUSHROOM:

In the present study, edible oyster mushroom *Pleurotus florida* and *Pleurotus ostreatus* were cultivated by using the standard procedure of Tamil Nadu Agricultural University.

(i) Experimental Design:

In the present study *Pleurotus florida* and *Pleurotus ostreatus* were cultivated by bag method using three different agrowaste as substrates.

- **Substrate A:** Paddy straw
- **Substrate B:** Sugarcane bagasse
- **Substrate C:** Banana leaf
- **Substrate D:** 1:1:1 ratio of paddy straw, sugarcane bagasse, banana leaf

(ii) Substrate Preparation:

- **Soaking:**

The selected agro-wastes (paddy straw, sugarcane bagasse and banana leaves) were cut into small pieces (6 -10cm) and soaked in water for 12 – 14 hours.

- **Sterilization:**

Soaked substrates were sterilized at 121℃ for 20 -30 minutes by using pressure cooker. After sterilization, the excess amount of water content was removed and cooled down by shade drying in room temperature.

- **Bag Preparation:**

Before starting, the hands were washed thoroughly using antiseptic lotion. Polypropylene bags with size 60 x 30 cm and with a thickness of 80 gauge were used for the cultivation. The bottom ends of the bags were tied using thread and turned to inside.

- **Layering of Substrate:**

The sterilized substrates were filled in the bag to a height of 3 inches. A handful of grain based spawns were sprinkled over the layer. Likewise, few layers were placed on the bag. Finally, the bag was pressed gently and tied with a thread. A few holes were made on the bags to facilitate ventilation and for the removal of excess water.

- **Spawn Running:**

The spawned bags were kept in a dark room for 1 week to facilitate the spawn running and colonization. Then the bags were transferred to cropping room.

- **Temperature and Humidity:**

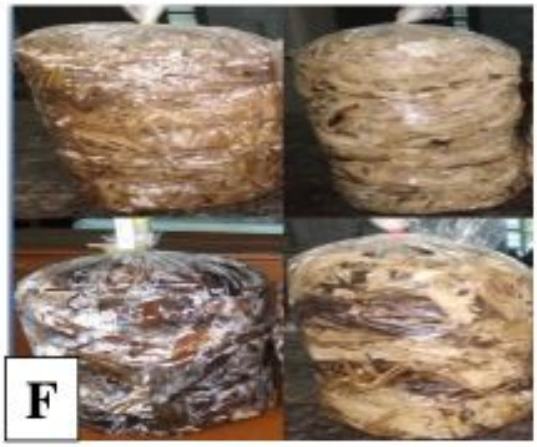
The spawned bags were kept under 22℃ - 25℃ temperature and required humidity of 85% was maintained by spraying water on the walls of the mushroom unit, four or five times in a day.

- **Harvesting:**

After colonization the mycelium started producing fruiting bodies. Initially it looked like a pin head and it was transformed to a full matured fruiting body within two days. After maturity the edges of the pileus starts to

Plate 2

PREPARATION OF BAGS FOR MUSHROOM CULTIVATION



A – Soaked paddy straw

B – Soaked sugarcane bagasse

C – Soaked banana leaves

D – Sterilization of Substrates

E – Packing of mushroom cultivation bags

F – Mushroom cultivation bags

Plate 3

MUSHROOM CULTIVATION ROOM

shrink towards inside. At this stage the fruiting bodies were collected manually and used for further experiments.

- **Data Collection:**

The bags were observed carefully from the preparation of bags till last day of harvest. Number of days required for colonization, pin head formation, fruiting body maturation and yield were recorded.

MORPHOLOGICAL PARAMETERS

Length and width of stipe and pileus were measured immediately after harvesting with the help of thread and measuring scale.

BIOLOGICAL EFFICIENCY (Carvalho *et al.*, 2012)

Biological efficiency is a term frequently used in the mushroom industry to describe the potential of the macro fungus to yield fruiting body (mushroom) from a known weight of substrate. Biological efficiency was calculated with the help of observed data.

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of the substrate}} \times 100$$

ORGANIC MASS LOSS (Carvalho *et al.*, 2012)

Organic mass loss of the substrate was calculated by using the following formula.

$$\text{Organic mass loss (\%)} = \frac{\text{Initial substrate dry mass} - \text{residual}}{\text{Initial substrate dry mass}} \times 100$$

IDENTIFICATION OF COMPETITOR MOULD

Mushroom beds were observed carefully from the 1st day until the last harvest to identify the competitor mould. Appearance of color change and undesirable odour indicates the microbial contamination. Such competitors were identified and isolated.

NUTRIENT CONTENT ANALYSIS

Moisture Content (Masamba and Kazombo-Mwale, 2010)

Known amount of sample was dried with the help of hot air oven at 105℃ for 12 hours.

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Total ash content (Gaur *et al.*, 2016)

One gram of dried mushroom powder of each species was weighed and kept in a crucible then heated over low flame first for complete charring and then placed in muffle furnace for about 4 - 5 hours at 600°C. It was weighed after cooling in desiccator. To ensure complete ash formation, the crucible was then heated in the muffle furnace for 1 hour, cooled and weighed. This was repeated till two consecutive weights were same and ash was almost white or grayish in colour. Total ash was calculated using the following equation:

$$\text{Ash content (g/100 g sample)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

SAMPLE PREPARATION FOR PHOTOCHEMICAL ANALYSIS:

The fruiting bodies were shade dried and powdered with the help of mixer grinder. The powdered sample was sieved to get uniform size particle and stored in an airtight container. The extract was prepared with the help of different solvents such as

hexane, chloroform, ethyl acetate, methanol, ethanol and water in 1:10 ratio (1gram sample in 10 ml solvent)

QUALITATIVE ANALYSIS

Phytochemical constituents were analyzed using hexane, chloroform, ethyl acetate, methanol, ethanol and water extracts of powderd fruiting bodies of *Pleurotus florida* and *Pleurotus ostreatus*. Standard procedures were followed **(Horbone1984, Kokate *et al*., 1995, Harborne, 1998).**

Test for Alkaloid (Wagner's test):

About 1 ml of extract was taken and few drops of Wagner's reagent was added and the formation of a reddish brown precipitate indicate the presence of alkaloids.

Test for Flavanoid (Shinoda Test):

About 1 ml of extract was added to pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid was added. Formation of pink color indicated the presence of Flavanoids.

Test for Phenol (Lead acetate test):

About 1 ml of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicated the presence of tannins and phenolic compounds.

Test for Tannin (Ferric chloride test):

About 1 ml of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black color indicated the presence of tannins.

Test for Steroid and Phytosteroid:

About 1 ml of extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. Appearance

of brown ring indicated the presence of steroids. Appearance of blush brown colour indicated the presence of phytosteroids.

Test for Carbohydrate (Benedict's test):

About 5 ml of Benedict's solution was added to 1 ml of extract and boiled in water bath. The appearance of red or yellow or green precipitate indicated the presence of reducing sugars.

Test for Saponin (Foam test):

About 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1cm indicated the presence of saponin.

Test for Glycoside:

About 0.5 ml of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicated the presence of glycosides.

Test for Protein & Amino Acid (Ninhydrin test):

About 0.5 ml of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicated the presence of proteins, peptides or amino acids.

Test for Terpenoid:

Five ml of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of terpenoids.

Test for Phlobatannin

Sample was boiled with 1% aqueous hydrochloric acid to produce red precipitate indicated the presence of phlobatannins.

Coumarin:

About 3 ml of 10% NaOH were added to 2 ml of plant extracts. The formation of a yellow colour was an indication for the presence of coumarins.

Cardiacglycoside (Keller-Killani Test):

Two ml of plant extract were treated with 2 ml glacial acetic acid containing a drop of $FeCl_3$. A brown coloured ring or brown-violet under a brown greenish layer indicated the presence of cardiac glycosides.

QUANTITATIVE ESTIMATION OF NUTRIENT COMPOSITION

Total Soluble Protein (Lowry *et al*., 1951)

Requirements:

- Alkaline copper reagent
- Solution A- 20% Sodium carbonate in 0.1 N sodium hydroxide
- Solution B- 1% Sodium potassium tartarate
- Solution C- 0.5% copper sulphate

To prepare 100 ml alkaline copper reagent, 98 ml of solution A, 1ml of solution B and 1 ml of solution C were mixed together freshly.

Folin-ciacalteau reagent (commercial reagent was diluted in the ratio of 1:1 with distilled water at the time of use).

Procedure:

100 mg of sample was homogenized in 10 ml of distilled water and filtered through a muslin cloth and centrifuged at 3000 rpm for 10 minutes. To the supernatant, 10% trichloro acetic acid (TCA) was added in 1:1 ratio and left in an ice bath for 30 minutes to precipitate protein. Then centrifuged at 3000 rpm for 5 minutes

and discarded the supernatant. The precipitate was dissolved in 0.1N sodium hydroxide and diluted to a known volume.

To 0.5 ml of protein extract, 5 ml of alkaline copper reagent was added. After thorough mixing, 0.5 ml of folinciocalteau reagent was added and allowed to stand for 30 minutes; the blue colour appeared and absorbance was measured at 650 nm using UV visible spectrophotometer (Model No: UV 2371). The analysis was performed in triplicates and the amount of protein was calculated and expressed as mg/g DW. Bovine serum albumin (BSA) was used as standard.

Estimation of Carbohydrates: Phenol-Sulpuric Acid Method (Dubois *et al.*, 1956)

Requirements:

- 5% phenol (5 ml phenol + 95 ml distilled water)
- 96% Sulphuric acid (96% sulpuric acid + 4 ml distilled water)

Procedure:

100 mg of sample was grounded with 10 ml distilled water. It was then filtered and centrifuged. The filtrate was collected. To 0.1 ml of the filtrate, 0.9 ml of distilled water, 1 ml of 5% phenol and 5 ml of 96% H_2SO_4 were added. After 30 minutes absorbance was measured at 490 nm using UV visible spectrophotometer (Model No: UV 2371). The analysis was performed in triplicates and the results were expressed as mg/g sample. Glucose was used as standard.

Estimation of Free Amino Acids (Moore and Stein, 1948)

Total free amino acids (ninhydrin method) were determined according to the procedure given by Moore and Stein.

Requirements:

- Ninhydrin reagent
- n-propanol

Procedure:

1 ml of the sample was mixed with 1 ml of Ninhydrin reagent in a test tube. Tubes were kept in boiling water bath for 20 minutes and then added 5 ml of diluent (equal volume of water and n-propanol) incubated at room temperature for 15 minutes and absorbance was read at 570 nm against a reagent blank. The analysis was performed in triplicates, and the results were expressed as mg/g sample.

Determination of Total Lipid (Folch *et al.*, 1957)

Requirements:

- $CHCL_3$
- CH_3OH
- H_2SO_4
- Phosphovanillin reagent

Procedure:

500 mg of dried sample was taken in a screw capped test tube, 10 ml of 2:1 $CHCL_3$:CH_3OH solvent mixture was added. The tube was loosely capped and heated in a water bath at 60ºC for 30 minutes. After cooling the solution, the volume was made up to 10 ml with the solvent mixture. 0.4 ml of the extract was pipetted in a separate test tube, allowed to dry completely and digested with 0.4 ml of concentrated H_2SO_4 by boiling in a water bath for 10 minutes. After cooling the tube, 5 ml of phosphovanillin reagent was added and allowed to stand for 30 minutes for colour

development. The absorbance was then measured at 520nm against a reagent blank using spectrophotometer (Model No: UV 2371). Cholesterol was used as standard.

DETERMINATION OF ANTIOXIDANTS

Estimation of Total Phenolic Content (Duan *et al*., 2006)

Requirements:

- 50% Folin – ciocalteau reagent (Folin – phenol)
- 20% Sodium carbonate

Procedure:

100 mg of plant material was homogenized with 10ml of distilled water and filtered through a muslin cloth. 1 ml of the filtrate was added to 1.5ml of de-ionized water and 0.5ml of 50% Folin – ciocalteau reagent and the content were mixed thoroughly. After one minute, 1 ml of 20% sodium carbonate solution was added and mixed. The blank contains all the reagents and solution except the sample. After 30 minutes of incubation at 37℃, the absorbance was measured at 750nm. Galic acid was used as standard.

Estimation of Total Flavonoid Content (Zhinshen *et al*., 1999)

Requirements:

- 5% Sodium nitrate
- 10% Aluminium chloride
- 1M Sodium hydroxide

Procedure:

100mg of sample was homogenized with 10ml of distilled water and filtered through a muslin cloth. 1ml of extract was added with 4ml of distilled water and

mixed. After 5 minutes, 0.3ml of 5% sodium nitrate was added and again after 5 minutes, 0.3ml of aluminium chloride was added. After 5 minutes, 2ml of 1M sodium hydroxide was added and final volume was made up to 10ml with distilled water. A blank was prepared by adding all the reagents except the sample. The absorbance was read at 510nm against blank. Quercetin was used as standard.

Estimation of Total Tannin Content (Julkunen – Titto, 1985)

Requirements:

- 4% Vanillin (prepare with methanol)
- Concentrated hydrochloric acid

Procedure:

100 mg of sample was homogenized with 10 ml of distilled water and filtered through a muslin cloth. 1ml of aliquot of aqueous extract was mixed with 3ml of 4% vanillin (prepare with methanol) and 1.5ml of concentrated HCL was added. The solution was shaken vigorously and left to stand at room temperature for 20 minutes in darkness. A blank was prepared by adding all the reagents except the sample. The absorbance was read at 500 nm. Tannic acid was used as a standard.

Determination of Vitamin C Content (Jagota and Dani, 1982)

Vitamin C estimation was determined following the Folin-ciocalteu reagent method by Jagota and Dani, 1982, with slight modifications.

Requirements:

- 10% trichloroacetic acid
- Folin-ciocalteu reagent

Procedure:

0.5 ml of the extracts was added to 0.8 ml of 10 % trichloro acetic acid and vigorously shaken and the mixtures were kept on ice for 5 minutes and then centrifuged at 3000 rpm for 5 minutes. This extract (0.2 ml) was then diluted to 2 ml with distilled water. Commercially prepared 2.0 M Folin-ciocalteu was diluted 10 fold with distilled water and 0.2 ml of this diluted reagent was added to the mixture and vigorously shaken. After 10 minutes at room temperature, the absorbance was measured at 760 nm against distilled water as a blank and the vitamin C content was estimated through the calibration curve of ascorbic acid.

Determination of Vitamin B_2 (Riboflavin) Content (Okwu and Emenike, 2006)

Requirements:

- 50 % Ethanol
- 30 % H_2O_2
- 40 % Sodium sulphate (made with ethanol)

Procedure:

About 5 g of the sample was extracted with 100 ml of 50% ethanol solution and shaken for 1 hour. This was filtered into a 100 ml flask. 10 ml of the extract was pipetted into 50 ml volumetric flask. 10 ml of 30% H_2O_2 were added and allowed to stand over in a hot water bath for about 30 minutes. 2 ml of 40% sodium sulphate (made with ethanol) were adding. This was made upto 50 ml mark and the absorbance measured at 510 nm in a spectrophotometer and vitamin B_2 content was estimated through the calibration curve of riboflavin dust.

DETERMINATION OF TRACE ELEMENTS (Agarwal *et al.,* 2011)

To determine heavy metal concentration, wet digestion method of the dried samples was adopted. 1 g of each air dried and sieved sample was ashed in a muffle furnace at 460ºC for 4 hrs. The ash was digested in 10 ml aquaregia (1 part conc. HNO_3 + 3 parts HCl) in a digestion tube on the heating blocks at different temperatures for a total of nine hours spreading over 2 h at 25ºC, 2 h at 60ºC, 2 h at 105ºC and 3h at 125ºC). After the digestion, the residue was transferred to a 100 ml volumetric flask. The clear solution was made up to the mark with double distilled water. A blank digestion solution was made for comparison. For calibration purpose, a standard solution for each element under investigation was prepared.

Analysis

Metallic concentrations were measured with atomic absorption spectrophotometer with double beam and deuterium background correction. Hollow cathode lamps of Pb, Cd, Zn, Co, and Cu were used at specific wave lengths.

FT – IR

Mushroom samples were lyophilized and mixed with KBr pellets and then subjected to FT-IR spectral analysis. The dried pellets were subjected to FT-IR spectroscopy measurement in the spectral range of 4000 – 400 cm with resolution of 4 cm. The results were compared with standard values and the functional groups were identified.

GC-MS ANALYSIS

GC-MS analysis of the mushroom samples were performed using a GC Clarus 500 Perkin-Elmer system comprising a AOC - 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite-1,

fused silica capillary column (330 mm × 0.25 mm ID × 1µm df, composed of 100% Dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/minute and an injection volume of 0.5 µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minute, to 200°C, then 5°C/minute to 280°C, ending with a 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. Total GC running time was 36 minutes. The relative percentage of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass. Interpretation on mass spectrum of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

SUBSTRATE ANALYSIS

The selected substrates were dried and made into powder using mixer grinder. It was used for further analysis.

pH analysis (Jackson, 1965)

The pH of the substrats was determined using digital pH meter. Ten gram of sample was taken in 100 ml distilled water and stirred continuously for 30 minutes

with a glass rod. The pH of the suspension was recorded after half an hour of settling by the pH meter pre calibrated using standard buffers of 4.0, 7.0 and 9.0 pH.

Estimation of cellulose (Updegraff, 1969)

Requirements:

- Nitric acid
- Acetic acid
- 67 % Sulphuric acid (67 ml H_2SO_4 + 33 ml H_2O)

Procedure:

About 100 mg of the sample was added to the mixture of nitric acid (15 ml) and acetic acid (85 ml), boiled in the water bath for 20 minutes, and centrifuged. The residue was dissolved in 10 ml of 67 % sulphuric acid and diluted to 20 ml. From the diluted sample, 1 ml of sample was drawn and used for the estimation of sugar by anthrone reagent and absorbance was measured using spectrophotometer at 620 nm.

Estimation of lignin (Chesson, 1978)

Requirements:

- Sulphuric acid
- Hydrochloric acid

Procedure:

One gram of the sample was added to 5 ml of concentrated sulphuric acid (95 - 98 %) + 50 ml hydrochloric acid (37 %) shaken well and kept for digestion for 16 hours. Later, the content was transferred to a 1,000 ml conical flask containing 450 ml of distilled water and was boiled for 10 minutes. The contents of the flask were filtered through G3 glass filter. The acidic residues were washed to neutrality with

distilled water, dried at 105°C for 24 hours and then weighed. The results were expressed in terms of percentage of lignin content on dry weight of the substrate.

Cellulose:Lignin (C:L)

C:L ratio was calculated from the estimation of cellulose and lignin contents.

Organic Carbon

The total organic carbon content was estimated using Walkey and Black method (1934) and it is calculated using the following formula:

% of oxidizable organic carbon = (Vol. of blank – Vol. of sample X 0.3 x Molarity)

% of total organic carbon (w/w) = 1.334 X % TOC

Estimation of Total Nitrogen (Linder, 1944)

5 ml aliquot of digested sample was taken separately in a 50 ml volumetric flask. 2 ml of sodium hydroxide was added to partially neutralized the excess acid and 1ml of 10% sodium silicate was added to prevent turbidity and samples were made up to the volume and were mixed. Then, 5 ml aliquot of sample was taken in a cuvette and 4 drops of Nessler's reagent was added and thoroughly mixed after the addition of each drop. The blank was also prepared following the same procedure. Absorbance was read at 540 nm in spectrophotometer. Ammonium sulphate was used as standard.

$$\text{Percent Nitrogen} = \frac{\text{C x ml of digest}}{\text{Aliquot taken (ml) x weight of sample (g)}} \times 100$$

C = concentration of N_2 in aliquot as read out from the standard curve.

DETERMINATION OF ANTIOXIDANT ACTIVITIES

Free Radical Scavenging Assay (Hatano *et al*., 1998)

Requirements:

- DPPH
- Methanol

Procedure:

Free radical scavenging assay was measured by 2-2 Diphenyl, l-picrylhydrazine (DPPH) method proposed by Hatano *et al*. (1998) with slight modifications. 1 ml aliquot of test sample was added to 0.004% DPPH solution prepared in methanol. The mixture was vortexed for 1 minute and kept at room temperature for 30 minutes in darkness. The absorbance was read at 517nm. A low absorbance of the reaction mixture indicated a high free radical scavenging activity. Ascorbic acid is used as a standard.

$$\text{DPPH Scavenging activity (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

A control is the absorbance of the DPPH solution without test solution. A test is the absorbance of DPPH with the test solution. Methanol was used as blank.

Ferric Ion Reducing Antioxidant Assay (Oyaizu, 1986)

Requirements:

- Phosphate buffer
- 10% TCA
- 0.1% Ferric chloride solution

Procedure:

1 ml of the test solution was mixed with 1 ml of phosphate buffer (0.2 M, pH 6.6) and incubated at 50ºC in a water bath for a minute. The reaction was stopped by adding 1ml of 10% TCA solution and then centrifuged at 5000 rpm for l0 minutes. The supernatant (1.5 ml) was mixed with 1.5 ml of distilled water and 0.1 ml of 0.1% Ferric chloride solution and allowed to stand for 10 minutes. The absorbance was measured at 700 nm and higher absorbance indicates greater reducing power. Ascorbic acid was used as control.

Hydrogen Peroxide Scavenging Activity (Chandrika *et al.*, 2007)

Requirements:

- Phosphate buffer
- H_2O_2
- Ascorbic acid

Procedure:

Samples were dissolved in 0.5 ml of 0.1 M phosphate buffer (pH 7.4), mixed with 0.5 ml of 20 mM H_2O_2 solution and measured at 230 nm. Ascorbic acid and phosphate buffer were used as positive and negative controls respectively. The activity was calculated according to the following equation:

$$H_2O_2 \text{ scavenging activity } (\%) = (1 - A_1/A_0) \times 100$$

Where A_1 is the absorbance of the test sample and A_0 is the absorbance of negative control.

ANTIBACTERIAL ASSAY

Bacterial cultures of *Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Salmonella paratyphi* and *Proteus vulgaris* were obtained from our department and were used for evaluating antibacterial activity. The bacteria were maintained on nutrient broth at 37ºC in incubator.

Preparation of Inoculum: The gram positive bacteria (*Bacillus subtilis*) and gram negative bacteria (*Klebsiella pneumoniae, Escherichia coli, Salmonella paratyphi, Proteus vulgaris*) were pre-cultured in nutrient broth overnight and incubated at 37ºC.

Disc Diffusion Method (Kirby *et al.*, 1986)

The antibacterial assay was done on human pathogenic bacteria such as *Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Salmonella paratyphi* and *Proteus vulgaris* by standard disc diffusion method. The cultures were spread on to nutrient agar plates using sterile cotton swabs. Sterile paper discs of 5 mm diameter with mushroom extract and standard antibiotic (Streptomycin 100 mg/ml) discs were placed over the inoculated plates followed by overnight incubation at 37ºC. The antibacterial activity was assigned by measuring the diameter of the zone of inhibition around the disc.

BIOSYNTHESIS OF SILVER NANOPARTICLES

The mushroom extraction was prepared by suspending about 10g of dry mushroom powder in 100ml of double distilled water and boiled at 60ºC temperature for 15 minutes. Then the solution was filtered through a Whatman filter paper No. 1 and the solution was used as stock solution for future experiment.

1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 2ml of mushroom extract was added into 10ml of 1mM silver nitrate solution. The reduction of silver nitrate occurred within 15min which resulted in the colour change of the solution into dark brown colour.

Characterization of Synthesized Silver Nanoparticles

UV-Visible Spectroscopy

The bio-reduction of silver ion in the solution was monitored using UV-Visible spectrophotometer (Deep vision UV-Visible spectrophotometer 2371) in the range of 350-550 nm.

PREPARATION OF FERMENTED LIQUID FERTILIZER FROM SPEND MUSHROOM SUBSTRATE (SMS):

After harvesting the mushroom, the substrate were dried and made into a power using mixer grinder. 10 gram of SMS powder was mixed with 100 ml of water in a conical flask. To this mixture 0.1 gram of yeast was added and closed. This mixture was shaken well frequently. After one week of anaerobic fermentation, it was filtered through Whatman filter paper. This liquid was sterilized with the help of hot air oven and sodium metabisulfite (0.1%) was added. This was stored in a normal room temperature for further studies.

Phytotoxicity Study Using SMS Liquid Fertilizer:

Green gram seed was selected for the study. Five concentrations of SMS liquid fertilizer were (1 %, 2 %, 3%, 4 % and 5 %) used, it was taken as treated and distilled water was used as control. The toxicology test was carried out in petriplates. The seeds were surface sterilized with 0.1% mercury chloride solution and rinsed thoroughly with double distilled water thrice. An equal number of seeds were kept in

labeled petriplates and treated with different concentrations of fermented liquid fertilizer prepared from spent mushroom substrate. Toxic effect was measured in terms of percentage of germination, root length and shoot length after 10 days of germination.

Germination Percentage

Seed germination study was carried out on 3rd day after sowing. From all the treatments, the number of seeds germinated were counted and recorded. Germination percentage was calculated by the following formula.

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Seedling Growth Analysis

Length of the root and shoot

The root and shoot lengths were taken on 5th and 10th days after sowing by selecting 3 seedlings at random from all the treatments. The average value was recorded in centimeters.

SURVEY STUDY

Survey was conducted to study the people's knowledge about the health benefits of mushroom and mushroom cultivation. A standard questionnaire was prepared to investigate on their food habits, availability of mushroom, price, available varieties, recycling of agricultural wastes, interest and knowledge about mushroom cultivation were asked during the survey. The responses were recorded and analyzed.

Statistical Analysis

Statistical analysis was carried out by using data analysis tool package of MS office Excel. The experiments were conducted in three replicates using the same treatments. The data of all values were statistically analyzed and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Balanced diet is the supporting treatment for the prevention of illness and especially against oxidative stress. In this context, mushrooms have long history of use in the oriental medicine to prevent and fight numerous diseases. Nowadays, mushroom extracts are commercially used as dietary supplements for their properties mainly for the enhancement of immune function. The present research work was planned to cultivate *Pleurotus* species and study the sustainable way for transformation of agro waste to potential nutritious food and fertilizer.

COLONISING PERIOD (SPAWN RUN)

Spawn run refers to the period during which mycelia spread and colonise the substrate so that it covers the whole substratum. It is a vegetative stage in the development of the fungi which requires specific conditions to be successful **(Oei, 1991).** For *Pleurotus* mushroom, spawn run duration differs depending on species type and substrate used. In the present study, three different substrates (paddy straw, banana leaves and sugarcane bagasse) were investigated to determine the growth and yield of two different species of *Pleurotus* namely *Pleurotus florida* and *Pleurotus ostreatus.* These substrates directly affect the time frame to attain the maximum mycelial growth and also take part in the yield attribute for oyster mushroom. Time required for completion of spawn run is presented in **Table 1.**

All mushroom bags showed mycelial growth and successful colonization of the substrate. Time required for completion of spawn running in *Pleurotus florida* and *Pleurotus ostreatus* varied on different substrates ranged from 15 to 17 days and

TABLE 1: EFFECT OF DIFFERENT SUBSTRATES ON THE GROWTH PERFORMANCE OF PLEUROTUS FLORIDA AND PLEUROTUS OSTREATUS

Species	Substrates	Spawn Running Days	Yield (gram)	No. of pinheads	Matured	Immature	Fruiting body Developed From Pinheads (%)	Biological Efficiency (%)	Organic Mass Loss (%)
P. florida	A	15.5	582.5	211.5	83	126	39.2	77. 7	26.9
	B	15.7	158.33	32	17.3	15.3	54.2	21.1	15.7
	C	17.3	621.67	109.7	80. 7	29	73.6	82.9	39.6
	D	16	460	136.7	92. 7	44	67.8	61.3	24.8
P. ostreatus	A	22.3	587. 7	51.3	34	17.3	66.2	78.4	26.3
	B	37.3	101. 7	12	7. 7	4.3	63. 9	13.6	12.5
	C	29	571	33	27. 7	5.3	83.8	76.1	24.1
	D	28. 7	525. 7	29.3	22	7.3	75	70.1	21.6

22 to 37 days respectively. In both the species, the lowest time required for the completion of spawn run was recorded in paddy straw (15 days in *Pleurotus florida* and 22 days in *Pleurotus ostreatus*). Longest time required for the completion of spawn run (17 days) was noticed in *Pleurotus florida* cultivated on banana leaves and in *Pleurotus ostreatus* (37 days) cultivated on sugarcane bagasse. Between the two species of *Pleurotus*, *Pleurotus florida* showed the fastest colonization of mycelia than *Pleurotus ostreatus*. Among the substrates used for the present study, colonization was fastest on paddy straw in both the species than other substrates.

Differences in spawn run durations among species were evident in the studies conducted by **Ashraf *et al.* (2013)** comparing three *Pleurotus* species (*Pleurotus* sajor – caju, *Pleurotus ostreatus* and *Pleurotus djmor*) on three different waste (cotton waste, wheat straw and paddy straw). Our findings in the present experiment are almost similar to the findings of **Lalithadevi and Many (2014)** who reported that spawn running day was between 16 – 25 days on paddy straw. The findings of the spawn run on sugarcane bagasse did not agree with the report of **Hossain (2017)** who stated that *Pleurotus ostreatus* completed the spawn run in 17 days on sugarcane bagasse. Increase in number of days for spawn running on lignocellulosic waste materials might be due to slow hyphal growth of mushroom on substrates **(Mandeel *et al.*, 2005).**

Mycelium development and colonization is the initial step which provides suitable internal environment for the development of basidiocarp. Thus, exponential growth of mycelium is a key feature in mushroom cultivation **(Sharma *et al.*, 2013).** The variation in the days might be due to the difference in the chemical constituents and C:N ratio of the substrates **(Bhatti *et al.*, 1987).** These results were similar to the

findings of **Vanathi *et al.*, (2016)** they have cultivated *Pleurotus florida* and reported 16 – 19 days for spawn running, it was highest in sugarcane trashes. **Iqbal *et al.*, 2016** reported that oyster mushroom cultivated upon sugarcane bagasse took 28.5 days for spawn running. The present study is corroborated with these findings. The occurrence of influential proportion of lignin, hemicellulose and alpha-cellulose in the growing medium was the assumed factor for higher rate of spawn running in banana leaves and rice straw substrate **(Mondal *et al.*, 2010).**

NUMBER OF PINHEADS AND PERCENTAGE OF FRUITBODIES DEVELOPED FROM PINHEADS

Pleurotus species produced significantly different numbers of pin heads on different substrates as shown in **Table 1.** In both the species, maximum numbers of pinheads (212 in *Pleurotus florida* and 51 in *Pleurotus ostreatus*) were recorded on paddy straw followed by pinheads developed on the mix of paddy straw + banana leaves + sugarcane bagasse in *Pleurotus florida* (137) and banana leaves (33) in *Pleurotus ostreatus* while minimum numbers of pinheads were observed on sugarcane baggase (32 in *Pleurotus florida* and 12 in *Pleurotus ostreatus*). Between the two species of *Pleurotus*, *Pleurotus florida* showed the highest number of pin heads (212) than *Pleurotus ostreatus* (51). From the present study, it was concluded that maximum numbers of pin heads were noticed on paddy straw in both the species than other substrates.

The percentage of fruit bodies developed from pin heads was very low (39%) on paddy straw though maximum numbers of pinheads produced on the same. In both the species, the highest percentage of fruit bodies developed from pin heads was maximum on banana leaves (74% in *Pleurotus florida* and 84% in

Pleurotus ostreatus) followed by the mix of paddy straw + sugarcane baggase + banana leaves (68% in *Pleurotus florida* and 75% in *Pleurotus ostreatus*). Highest percentage of fruitbodies developed from banana waste may be due to the high-water holding capacity **(Plate 4-7)**.

Our findings are further supported by **Hague (2004)** and **Al Amin (2004)** who reported that highest number of pinheads of Oyster mushroom was found on paddy straw. Minimum numbers of pinheads were observed on sugarcane bagasse (12). Almost similar results are reported by **Hasan *et al.* (2015)** who observed minimum number of pinheads of oyster mushroom on sugarcane bagasse. The results were in accordance with the findings of **Al Amin (2004)** who reported maximum number of primordia and fruiting bodies of oyster mushroom on paddy straw. Formation of higher number of fruiting bodies may be due to the occurrence of glucose, fructose and trehalose in the substrate (**Kitamoto *et al.*, 1995). Poppe (1973)** reported that presence of Indole Acetic Acid (IAA) induces the formation of maximum fruiting body of mushroom.

EFFECT OF SUBSTRATE ON LENGTH AND WIDTH OF PILEUS AND STIPE

Among mushroom quality characteristics, pileus diameter, stipe length, stipe diameter are very important attributes **(Mondal *et al.*, 2010). Figure 1a** represents length and width of pileus and stipe of *Pleurotus florida* on different substrates. Maximum length of pileus and stipe (7.3 ± 0.8 cm and 3.1 ± 0.6 cm) was obtained on paddy straw while maximum width of pileus and stipe (8.4 ± 1.8 cm and 1.8 ± 0.1 cm) was noticed on sugarcane baggase and the mix of paddy straw + sugarcane baggase + banana waste respectively.

Plate 4

DEVELOPMENT OF FRUITING BODIES OF PLEUROTUS FLORIDA ON PADDY STRAW

DEVELOPMENT OF FRUITING BODIES OF PLEUROTUS FLORIDA ON SUGARCANE BAGASSE

Plate 5

DEVELOPMENT OF FRUITING BODIES OF PLEUROTUS FLORIDA ON BANANA LEAVES

DEVELOPMENT OF FRUITING BODIES OF PLEUROTUS FLORIDA ON SUBSTRATE MIX

Plate 6

DEVELOPMENT OF FRUITING BODIES OF PLEUROTUS OSTREATUS ON PADDY STRAW

DEVELOPMENT OF PLEUROTUS OSTREATUS FRUITING BODIES ON SUGARCANE BAGASSE

Plate 7

DEVELOPMENT OF FRUITING BODIES OF PLEUROTUS OSTREATUS ON BANANA LEAVES

DEVELOPMENT OF PLEUROTUS OSTREATUS FRUITING BODIES ON SUBSTRATE MIX

The data regarding the length and width of pileus and stipe of *Pleurotus ostreatus* is shown in **Figure 1b**. In the present study, maximum length and width of pileus was obtained (11.3 ± 2.2 cm and 21.5 ± 6.7 cm) on banana leaves followed by the mix of paddy straw + sugarcane bagasse + banana leaves (11.3 ± 3.4 cm and 20.3 ± 6.1 cm) respectively. The minimum length and width of pileus was noted (5.8 ± 0.8 cm and 8.2 ± 2.9 cm) on sugarcane bagasse. Our results are in consistence with the findings of **Sardar *et al.* (2016)** who observed minimum diameter of pileus (4.10 ±0.07 cm) on sugarcane bagasse.

Stipe length and width of *Pleurotus ostreatus* was observed on different substrates in the present study and found significant difference on different substrate is shown in **Figure 1b**. Maximum length of stipe (3.1 ± 1.5 cm) was obtained on the mix of paddy straw + sugarcane bagasse + banana leaves and paddy straw alone (3.0 ± 1.5 cm). Similarly, Maximum width of stipe (2.6 ± 1.1 cm) was obtained paddy straw alone and on the mix of paddy straw + sugarcane bagasse + banana leaves (2.3 ± 0.7 cm). Minimum length of stipe (1.3 ± 0.3 cm) was observed on banana leaves while the minimum width was noticed (1.2 ± 0.3 cm) on sugarcane bagasse.

Between the two species of *Pleurotus*, *Pleurotus ostreatus* showed the maximum length and width of pileus than *Pleurotus florida*. From the present study, it was concluded that maximum length and width of pileus were noticed on banana waste and the mix of paddy straw + sugarcane bagasse + banana waste than other substrates. Oyster mushroom quality depends on the length of stipe. **Mondal *et al.* (2010)** found that the higher the stipe length, the poorer the quality of the mushroom. Hence growers should use substrates that do not promote excessive growth of stipe length at the expense of marketable yield.

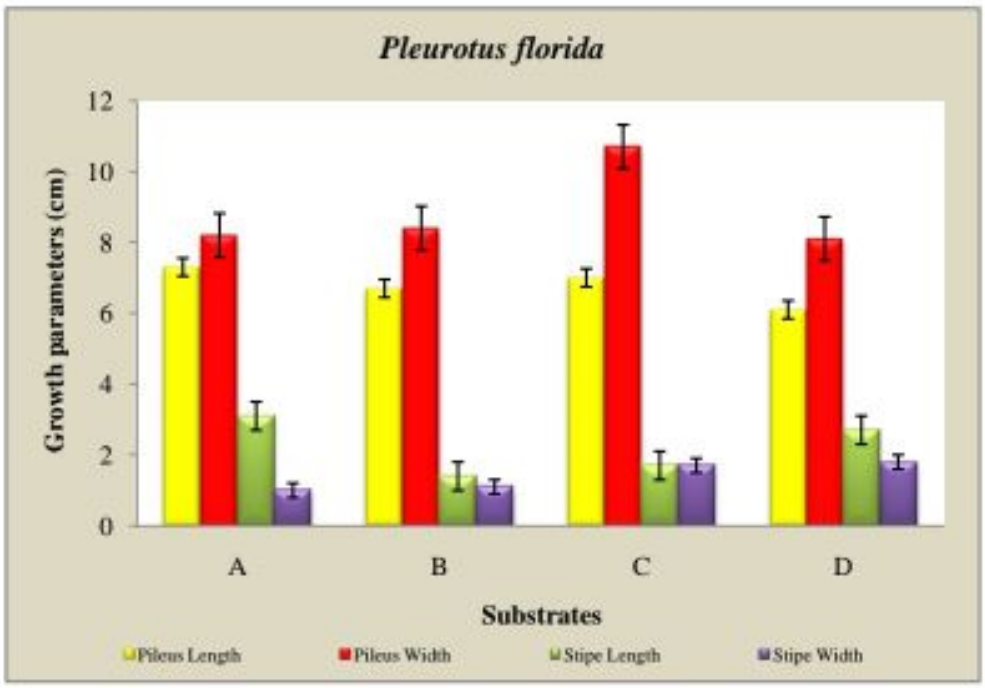


Figure 1a: Effect of different substrates on the growth parameters of *Pleurotus florida*

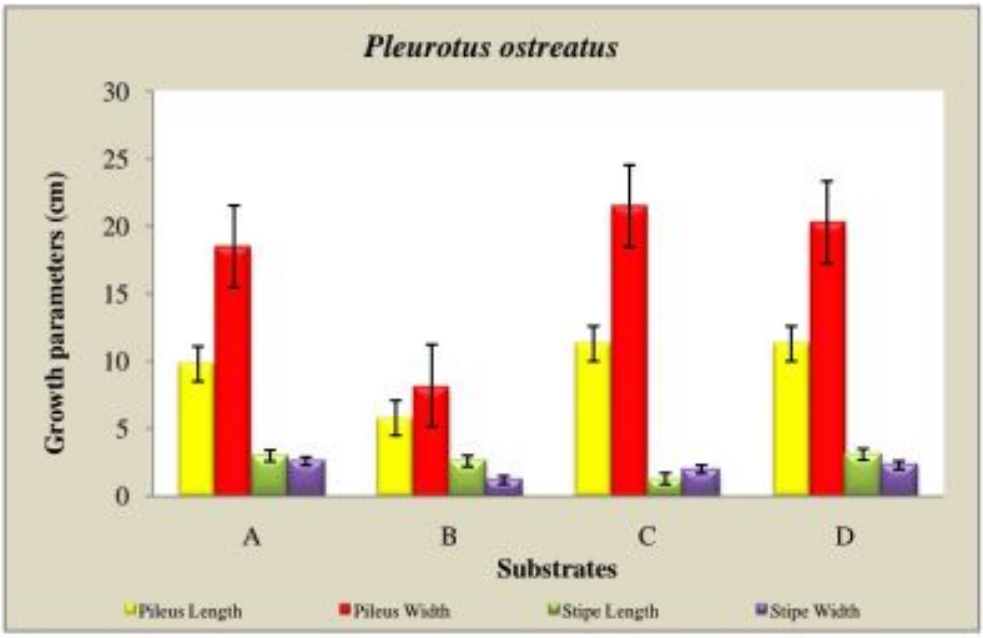


Figure 1b: Effect of different substrates on the growth parameters of *Pleurotus ostreatus*

A – Paddy straw **B – Sugarcane bagasse**

C – Banana leaves **D – Substrate Mixture**

The size of the fruiting bodies is depended on the water holding capacity of the substrate **(Chukwurah *et al.*, 2013)** and environmental conditions **(Sanchez, 2004). AMGA (2004)** identified temperature, relative humidity, fresh air and compact material as the major external factors that affect stalk length, stalk width and mushroom cap shape. The quality of oyster mushrooms relies upon its stalk length, higher the stalk length lesser will be the mushroom quality **(Zadrazil, 1978).**

TOTAL YIELD (g)

The yield pattern of *Pleurotus* species was observed and recorded in **Table 1**. The present study confirmed that the use of different substrates brought about a significant effect on yield of *Pleurotus florida* and *Pleurotus ostreatus*. In *Pleurotus florida*, the harvest yield ranged from 158g to 622g while in *Pleurotus ostreatus*, the harvest yield ranged from 102 g to 588 g **(Table 1).** From the present study, it was concluded that there was a difference in the yield between the selected *Pleurotus* species however, the difference is not significant. In *Pleurotus florida*, the average yield of mushroom fruitbodies was highest on banana leaves (622 g) followed by mushroom fruitbodies cultivated on paddy straw (583 g) while in *Pleurotus ostreatus*, the average yield of mushroom fruitbodies was maximum on paddy straw (588 g) followed by mushroom fruitbodies cultivated on banana leaves (571 g). In both the species, minimum yield was obtained in mushroom fruitbodies cultivated on sugarcane bagasse (102 g in *Pleurotus ostreatus* and 158 g *Pleurotus florida*). The increase in the yield of *Pleurotus florida* and *Pleurotus ostreatus* on paddy straw is due to easier way of getting sugars from cellulosic substances **(Ponmurugan *et al.*, 2007).** Superiority of paddy straw over other substrates in cultivation of *Pleurotus* species with respect to yield has been

reported earlier by **Pala *et al.* (2012).** Our results also agree with the result of **Ragunathan *et al.* (1996)** who reported that maximum yield was obtained by cultivation *P. sajor – caju* on paddy straw.

BIOLOGICAL EFFICIENCY (%)

Table 1 represents the variation for biological efficiency of *Pleurotus* species on different substrates. The highest percentage biological efficiency of *Pleurotus florida* was found on banana waste (82.9%) followed by paddy straw (77.7%) while in *Pleurotus ostreatus* the highest percentage biological efficiency was noticed on paddy straw (78.4%) followed by banana waste (76.1%). Our results also agree with the results of **Kumar *et al.* (2009)** who reported that the highest percentage biological efficiency of *Pleurotus florida* was recorded on paddy straw (77%). Higher the biological efficiency of different substrates represents its higher suitability for the cultivation of mushroom. The lowest biological efficiency (21.1% in *Pleurotus florida* and 13.6% in *Pleurotus ostreatus*) was obtained on sugarcane bagasse as shown in **Table 1.** Our results agree with the result of **Sardar *et al.* (2016)** who reported that lowest biological efficiency was obtained on sugarcane bagasse.

PERCENTAGE OF BIOMASS LOSS

The mushroom has the ability to degrade lignocellulosic materials during the idiophase stage following severe nitrogen and carbon depletion **(Manson *et al.*, 1989).** In *Pleurotus ostreatus*, biomass loss was maximum (26.3%) in paddy straw while in *Pleurotus florida*, biomass loss was maximum in banana waste (39.6%) which shows that degradation and solubilization was more intensive in the paddy waste and banana waste **(Table 1).**

SYMPTOMATOLOGICAL OF FUNGAL COMPETITOR IN OYSTER MUSHROOM CULTIVATION BAGS

Different fungi occurring in the substrate and competing with mushroom mycelium for space and nutrition are known as competitor moulds. In the present study, the specimen was observed in small clusters on mushroom cultivation bags filled with sugarcane bagasse. The fruitbody has distinctive reddish-brown sporangia supported on slender stalks **(Plate 8 – 9).** The specimen of the present study was identified upto the genus level as *Stemonitis* based on the characters described for *Stemonitis* from other parts of the world including India **(Hatano, 2007; Kharat and Nanir, 2010; Vasava *et al*., 2015; Ranade and Ranadive, 2016).** It is a common genus found associated with decaying wood and can be distinguished by its bright rusty brown coloured fruitbody, occurring small or medium sized clusters **(Phate and Mishra, 2014).** In the study, it was observed that the mould appeared only after the first yield and after that the yield was reduced drastically. The growth parameters were also reduced in both the species of *Pleurotus* cultivated on the same substrate compared to other substrates.

PRELIMINARY PHYTOCONSTITUENTS ANALYSIS

Table 2 and 3 show the qualitative analysis of *Pleurotus* species. Phytochemical analysis of both the *Pleurotus* species disclosed the presence of alkaloid, protein, carbohydrate, saponin, phenol, tannin and flavonoid in both the aqueous and alcoholic extracts. Terpenoid was present only in alcoholic extracts (methanol and ethanol) and glycoside was present only in ethanol extract of both the *Pleurotus* species. Steroid, phytosterol, quinone and phlobatannin were not detected in all the six extracts. Among the solvents used for extraction, the extract obtained

Plate 8

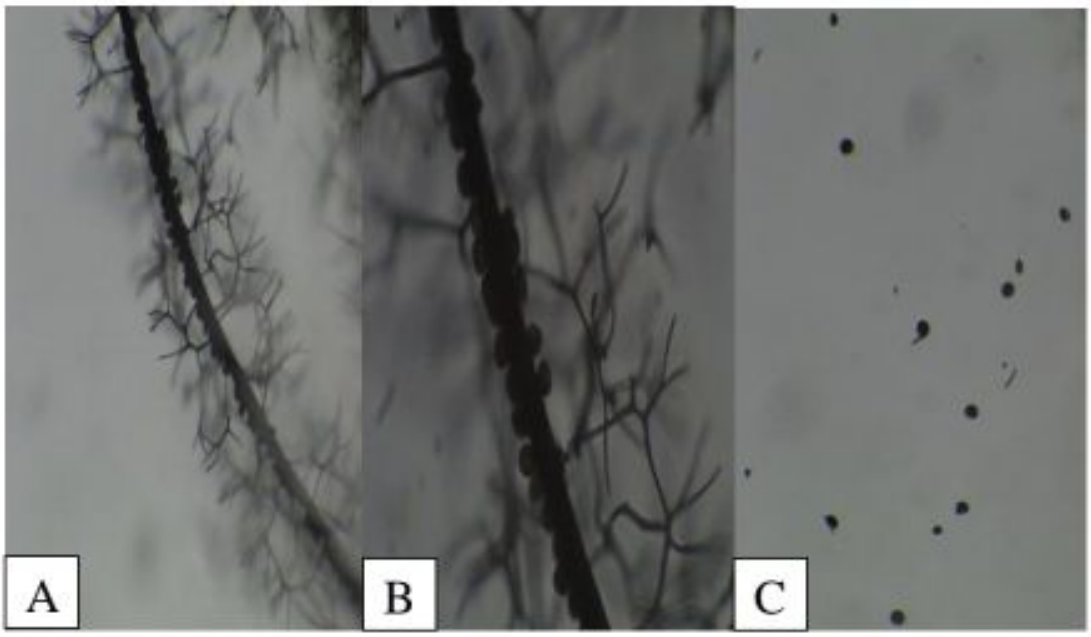
COMPETITOR MOULD IDENTIFIED FROM MUSHROOM CULTIVATING BAGS

STEMONITIS SPECIES 1

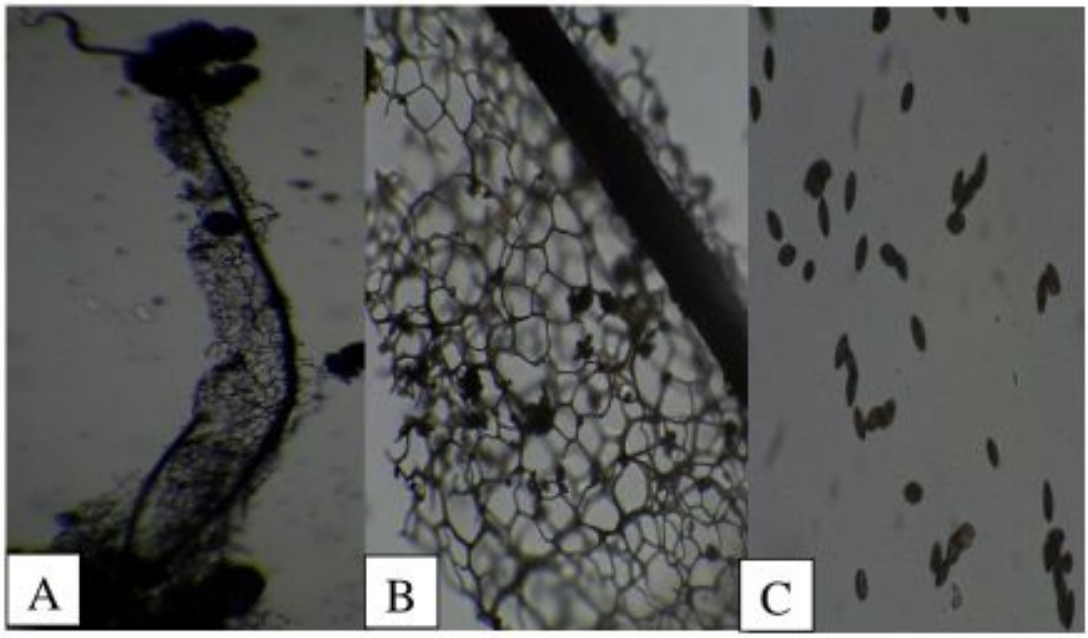
STEMONITIS SPECIES 2

Plate 9

MICROSCOPIC STRUCTURE OF STEMONITIS SPECIES 1



MICROSCOPIC STRUCTURE OF STEMONITIS SPECIES 2



A – Sporangium B – Spore arrangement C – Spore

TABLE 2: QUALITATIVE ANALYSIS OF PLEUROTUS FLORIDA CULTIVATED UPON DIFFERENT SUBSTRATES

Test	Hexane				Chloroform				Ethyl acetate				Ethanol				Methanol				Water			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Alkaloid	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Protein	-	-	-	-	+	+	+	+	-	-	-	-	++	++	++	++	+	+	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+	++	++	++	++	++	++	++	++	+	+	+	+
Glycoside	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-				
Saponin	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Tannin	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoid	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Steroid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phytosterol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpinoid	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-
Cardioglycoside	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-
Coumarin	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Phlobatannin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

'+' Indicates Presence '–' Indicates Absence

TABLE 3: QUALITATIVE ANALYSIS OF PLEUROTUS OSTREATUS CULTIVATED UPON DIFFERENT SUBSTRATES

Test	Hexane				Chloroform				Ethyl acetate				Ethanol				Methanol				Water			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Alkaloid	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Protein	-	-	-	-	+	+	+	+	-	-	-	-	++	++	++	++	+	+	+	+	+	+	+	+
Carbohydrate	-	-	-	-	+	+	+	+	+	+	+	+	++	++	++	++	++	++	++	++	+	+	+	+
Glycoside	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Saponin	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Phenol	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Tannin	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoid	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Steroid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phytosterol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpinoid	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-
Cardioglycoside	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-
Coumarin	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Phlobatannin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

'+' Indicates Presence '–' Indicates Absence

using ethanol showed a greater number of phytoconstituents as compared to others. Each individual species has their own specific mechanisms for the synthesis and metabolism of phytochemicals (**Obadai *et al.*, 2014**). Similarly, the oyster mushroom extract showed the occurrence of rich source of components such as alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds and polyphenols except phlobatannins, anthraquninones and hydroxymethyl anthraquinones **(Akyuz *et al.*, 2010).**

NUTRITIONAL VALUE

The nutritional value of edible mushroom is due to their high protein, fibre, vitamin, mineral contents and low - fat levels **(Mattila *et al.*, 2001; Barros *et al.*, 2008).** They are very useful for vegetarian diets because they provide all the essential amino acids for adult requirements; also, mushrooms have higher protein content than most vegetables. Besides, edible mushroom contains many different bioactive compounds with various human health benefits **(Gruen and Wong, 1982).** Several nutritional parameters were measured for both the *Pleurotus* species.

Moisture Analysis

Moisture percentage in mushroom depends on the maturity of fruiting bodies, species and storage conditions during packaging or processing. The result of moisture content of both the species of *Pleurotus* is presented in **Figure 2a**. The present study revealed that the highest moisture content was observed in sugarcane bagasse substrate in both the species of *Pleurotus* (91.3% in *Pleurotus florida* and 90.05% in *Pleurotus ostreatus*). Mushroom typically have a high moisture content that accounts for their short shelf life as they deteriorate if preservative measures aren't used after

harvest **(Adedayo Rachel, 2011).** Fresh mushroom contains regarding 90% moisture and 10% dry matter and dry mushroom contains 90% dry matter and 10% moisture **(Johnsy and Davidon, 2011). Ahmed *et al.*, (2009)** reported that little variation in the moisture content of *Pleurotus florida* cultivated upon different substrates. It slightly varied between 89.4 to 92.4% and clearly indicates that the moisture content is independent from the substrate.

Ash Content

Ash content of tested mushrooms was recorded between 84 mg/g DW to 98 mg/g DW in *Pleurotus florida* and 93 mg/g DW to 107 mg/g DW in *Pleurotus ostreatus* **(Figure 1b).** The highest ash content was ascertained on sugarcane bagasse in both the species of *Pleurotus* whereas the lowest was found on paddy straw in both the species. Between the two species of *Pleurotus*, *Pleurotus ostreatus* showed the maximum amount of ash content than *Pleurotus florida*. Ash contents can affect human mineral intake and these minerals of mushrooms were bioavailable **(Dikeman *et al*., 2005).**

Carbohydrate

Carbohydrate is a vital constituent of mushrooms of top quality. Carbohydrate act as an associate energy supplier and they are found in several foodstuffs. Carbohydrates are found in high proportions in edible mushrooms, including chitin, glycogen, trehalose and mannitol, besides, they contain fiber, β glucans, hemicellulose and pectic substances. Additionally, glucose, mannitol and trehalose are abundant sugars in cultivated edible mushrooms but fructose and sucrose are found in low amounts **(Zhang *et al*., 2008).**

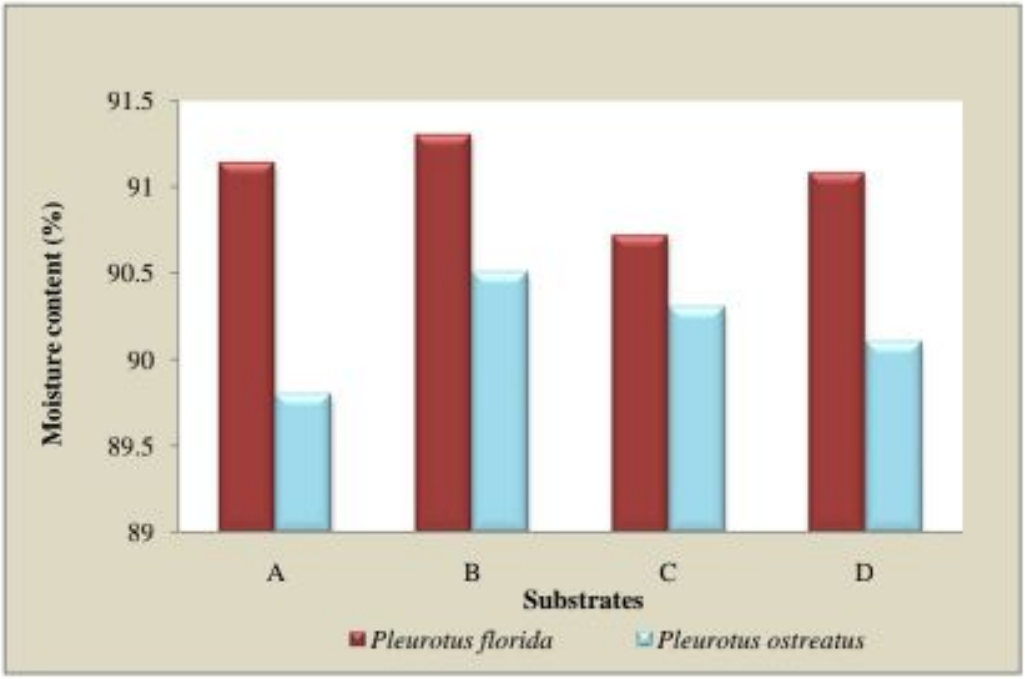


Figure 2a: Effect of different substrates on the moisture content of *Pleurotus florida* *Pleurotus ostreatus*

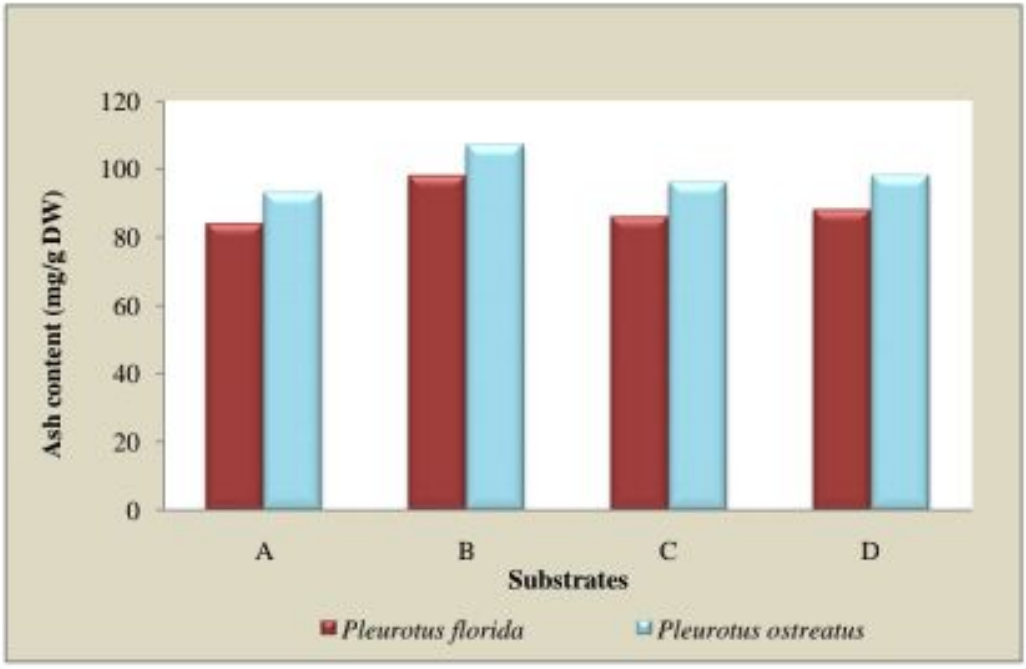


Figure 2b: Effect of different substrates on the ash content of *Pleurotus florida* *Pleurotus ostreatus*

A – Paddy straw **B – Sugarcane bagasse**

C – Banana leaves **D – Substrate Mixture**

The carbohydrate content of aqueous extracts of *Pleurotus florida* varied from 4.4 ± 0.03 mg /g DW to 7.8 ± 0.07 mg/g DW while in *Pleurotus ostreatus*, the carbohydrate content ranged from 3.0 ± 0.03 mg /g DW to 3.7 ± 0.02 mg/g DW. The carbohydrate content of ethanolic extracts of *Pleurotus florida* varied from 5.8 ± 0.03 mg /g DW to 9.0 ± 0.06 mg/g DW while in *Pleurotus ostreatus*, the carbohydrate content ranged from 5.6 ± 0.05 mg /g DW to 11.7 ± 0.02 mg/g DW **(Figure 3a).**

The results indicated that the highest amount of carbohydrate was found in ethanolic extract (9.0 ± 0.06 mg/g DW in *Pleurotus florida* and 11.7 ±0.02 mg/g DW *Pleurotus ostreatus*) of both the species of *Pleurotus* cultivated on sugarcane bagasse. Between the two species of *Pleurotus*, *Pleurotus ostreatus* had the maximum amount of carbohydrates than *Pleurotus florida.* Between the solvents used for the present study, ethanolic extract showed the maximum amount of carbohydrate than the aqueous extract.

Protein

Protein is an important constituent of dry matter of mushrooms. Protein content of mushrooms depends on the composition of substratum, size of pileus, harvest time and species of mushrooms **(Bano and Rajarathnam, 1982).** Protein content of *Pleurotus ostreatus* and *Pleurotus florida* was found maximum in sugarcane bagasse treated (194 ± 2.4 mg/g DW and 165.4 ± 0.4 mg/g DW) followed by the mix of paddy straw + sugarcane bagasse + banana leaves (184 ± 0.7 mg/g) in *Pleurotus ostreatus* and banana waste in *Pleurotus florida* (161 ± 0.5 mg/g DW) while the fruit bodies developed from paddy straw had the lowest protein content

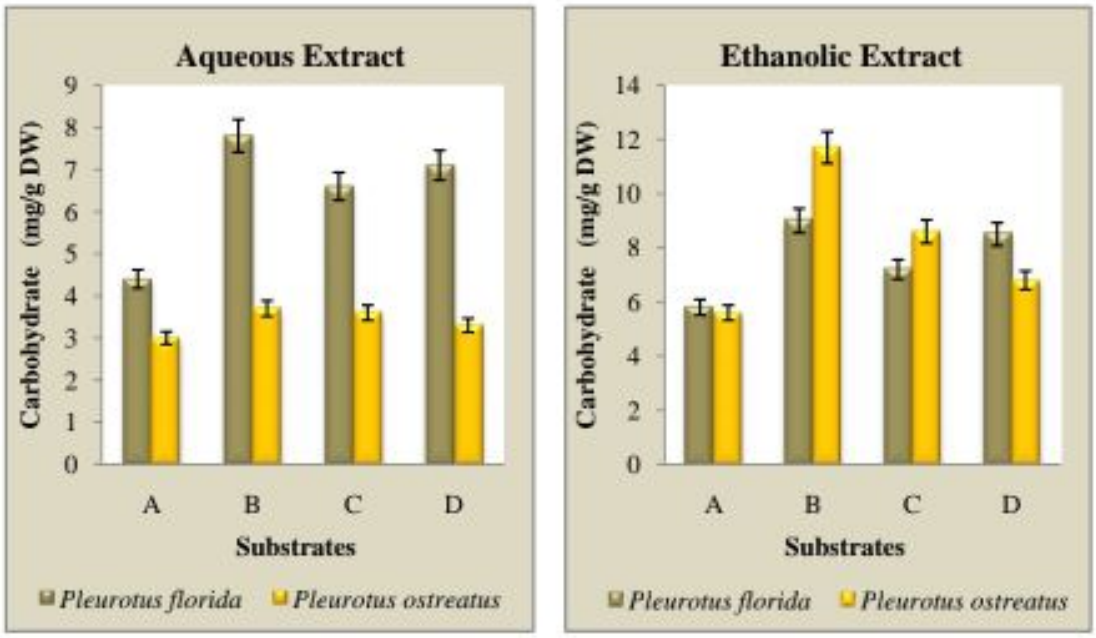


Figure 3a: Effect of different substrates on the carbohydrate content of *Pleurotus florida* and *Pleurotus ostreatus*

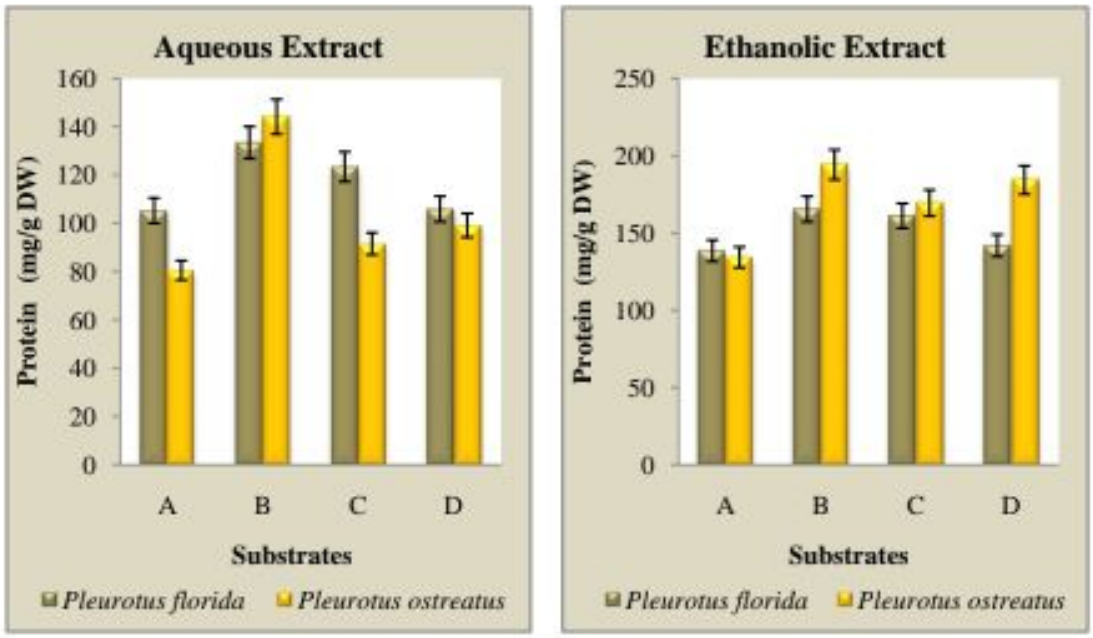


Figure 3b: Effect of different substrates on the protein content of *Pleurotus florida* and *Pleurotus ostreatus*

A – Paddy straw **B – Sugarcane bagasse**

C – Banana leaves **D – Substrate mixture**

(134 ± 2.27 mg /g DW in *Pleurotus ostreatus* and 138.4 ± 0.5 mg/g DW in *Pleurotus florida*) in both the species of *Pleurotus* **(Figure 3b).**

Protein content of the mushroom mycelium can be controlled by the amount of nitrogen supplied in the growth media. The Carbon: Nitrogen influences the protein and the fat content in the mushroom mycelium **(Shah *et al.*, 2004)**. They differ according to the species but this difference depends on the substratum, atmospheric conditions, age and part of the fruitification**.** *Pleurotus* species is an abundant source of superior quality protein with well distributed essential amino acids **(Patil *et al.*, 2010).** Protein deficiency is the world's most serious human nutritional problem. Mushroom is a promising food which can help to overcome protein deficiency problem **(Alam *et al.*, 2008).** Mushroom proteins are easy to digest. Protein occupies 20 and 40% on dry weight basis which is superior to many legumes like peanuts, soybeans and protein-yielding vegetable **(Chang and Mshigeni, 2001).**

Amino Acid

The mushroom protein is known to contain almost all the essential amino acids. Apart from essential amino acids, considerable amount of alanine, arginine, glycine, histidine, glutamic acid, aspartic acid, proline and serine can be found in mushroom. The free amino acid content of aqueous extract of dry thallus of *Pleurotus ostreatus* and *Pleurotus florida* ranged from 136.6 ± 1.02 mg/g DW to 155.9 ± 4.7 mg/g DW and 79.9 ± 1.2 mg /g DW to 124.5 ± 0.9 mg/g DW respectively **(Figure 4a).** The highest amount of amino acids was recorded in ethanolic extract of dry thallus of *Pleurotus ostreatus* (243.8 ± 1.17 mg/g DW) and *Pleurotus florida* (134.3 ± 0.58 mg/g DW) cultivated on the mix of paddy straw + sugarcane bagasse + banana waste while the lowest amount of amino acids was

registered in ethanolic extract of dry thallus of *Pleurotus ostreatus* cultivated on paddy straw and dry thallus of *Pleurotus florida* cultivated on sugarcane bagasse.

The quantitative spectrum of essential amino acids has served as the basis to calculate biological value, nutritional value and protein score **(Haque, 1989).** Mushroom provides all the essential amino acids required for the human diet and it is especially rich in leucine and lysine which are absent in most staple cereal crops **(Sadler, 2003; Chang and Buswell, 1996).**

Lipid

Lipid content of all the four investigated substrates showed variation **(Figure 4b).** In mushrooms, the fat content is very low as compared to proteins. In both the species of *Pleurotus*, The highest lipid content was found in ethanolic extract of fruit bodies developed on sugarcane bagasse (8.6 ± 0.07 mg/g DW in *Pleurotus ostreatus* and 10.1 ± 0.11 mg/g DW in *Pleurotus florida*) followed by the mix of paddy straw + sugarcane bagasse + banana leaves (7.8 ± 0.09 mg/g DW in *Pleurotus ostreatus* and 9.3 ± 0.09 mg/g DW in *Pleurotus florida*) while the ethanolic extract of fruit bodies developed from paddy straw showed the lowest lipid content (5.3 ± 0.06 mg/g DW in *Pleurotus ostreatus* and 6.3 ± 0.05 mg/g DW in *Pleurotus florida*).

Yilmaz *et al.* (2006) and **Pedneault *et al.* (2006)** reported that fat fraction in mushrooms is mainly composed of unsaturated fatty acids. Mushrooms are low in lipid content it possesses high concentration of polyunsaturated fatty acids nearly 72 to 85% in total fat content. The dominance presence of linoleic acid made mushroom as a health food **(Sadler, 2003; Chang and Mshigeni, 2001)**.

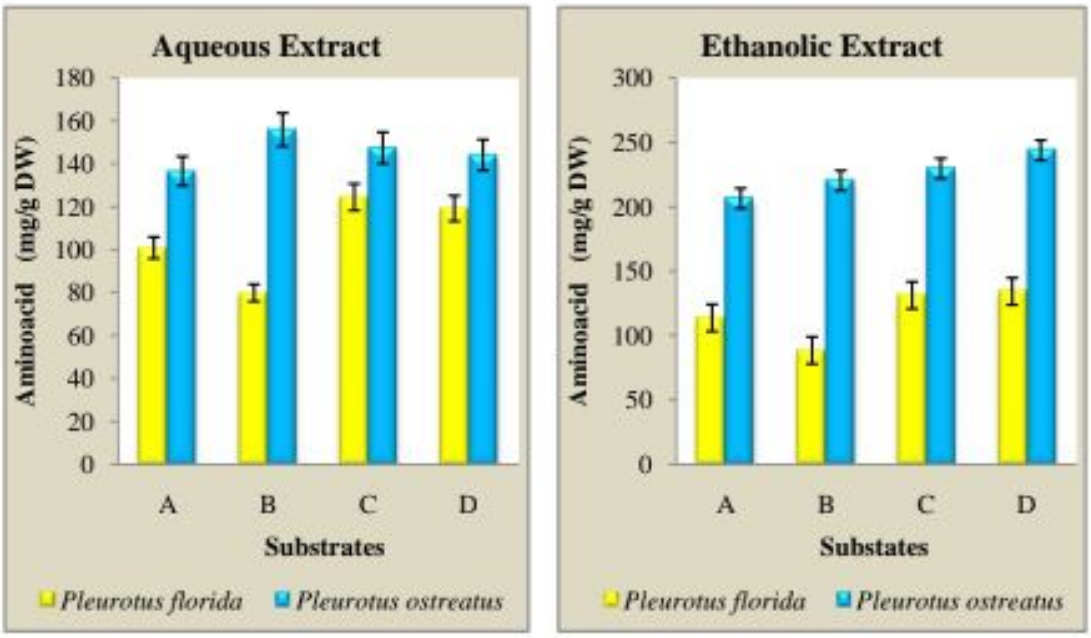


Figure 4a: Effect of different substrates on the aminoacid content of *Pleurotus florida* and *Pleurotus ostreatus*

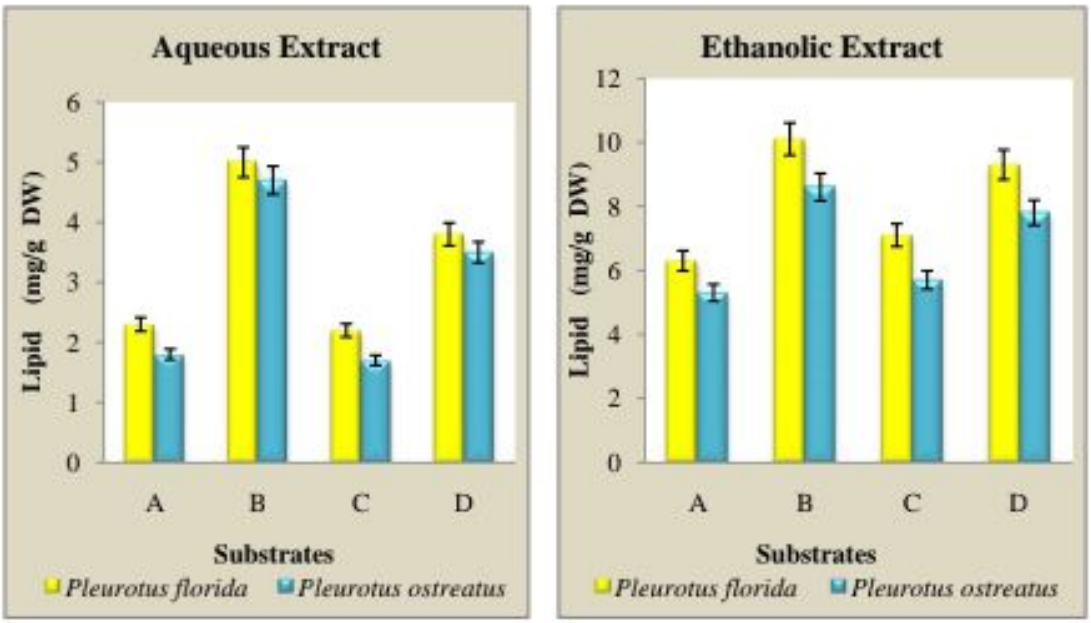


Figure 4b: Effect of different substrates on the lipid content of *Pleurotus florida* and *Pleurotus ostreatus*

A – Paddy straw **B – Sugarcane bagasse**

C – Banana leaves **D – Substrate mixture**

ANTIOXIDANT COMPOMUNDS

Total Phenolic Compounds (TPC)

The contents of total phenolic compounds in the mushroom extracts are shown in **Table 4.** The results expressed as milligrams of gallic acid equivalents per gram of dried mushroom (mg GAE/g DW). Total phenolic content of aqueous extract of *Pleurotus florida* varied from 1.57 ± 0.2 mg/g DW to 4.54 ± 0.12 mg/g DW while in *Pleurotus ostreatus*, total phenolic content ranged from 2.20 ± 0.15 mg/g DW to 4.09 ± 0.17 mg/g DW. The results indicated that the highest amount of total phenolic compound was found in ethanolic extract (7.64 ± 0.18 mg/g DW) of *Pleurotus florida* cultivated on sugarcane bagasse while in *Pleurotus ostreatus,* the highest phenolic compounds was obtained in ethanolic extracts (6.88 ±0.26mg/g DW) on paddy straw. Between the two species of *Pleurotus*, *Pleurotus florida* had the maximum amount of phenolic compounds than *Pleurotus ostreatus*. Among the substrates used for the present study, phenolic compound was found to be maximum on sugarcane bagasse in both the species than other substrates.

Phenolic compounds are a large group of secondary plant metabolites which play a major role in the protection of oxidation processes. Phenolic compounds have antioxidant properties and can act as free radical scavengers, hydrogen donators and singlet oxygen quenchers **(Croft, 1999).** Numerous studies have conclusively demonstrated that mushrooms also contain many phenolics which are important plant constituents because of their scavenging ability **(Elmastasa *et al.*, 2007; Turkoglu *et al.*, 2007).** In addition, phenolics exhibit a wide range of biological effects including antibacterial, anti-inflammatory and anti-hyperglycemic **(Liu *et al.*, 2012).**

Total Flavonoid Content (TFC)

Table 4 also shows the total flavonoid content of the samples with two different solvent extraction. Total flavonoid content of aqueous extract of *Pleurotus florida* varied from 2.38 ± 0.15 mg/g DW to 3.59 ± 0.11 mg/g DW while in *Pleurotus ostreatus*, total flavonoid content ranged from 1.69 ± 0.18 mg/g DW to 3.53 ± 0.42 mg/g DW. Relating to the ethanolic extraction, flavonoid content was higher in both the species of *Pleurotus* cultivated on sugarcane bagasse (4.4 mg/g DW) followed by banana waste (4.13 mg/g DW in *Pleurotus florida* and 4.08 mg/g DW in *Pleurotus ostreatus*).

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. A higher content of flavonoid was found in ethanol extract compared to aqueous extract. These results are consistent with previous report. A report by **Yeh *et al.* (2011)** stated that flavonoid content in ethanol extract of *Grifola frondosa* has been found to have significantly higher than cold and hot water extract. This is because the groups such as phenolic acids, lignin, flavonoids with structures containing – OH and – COOH functional groups are easily extracted by the polar solvent in samples.

Total Condensed Tannin (TCT)

The results of total condensed tannin of the mushroom extracts are shown in **Table 4.** Total tannin content of aqueous extract of *Pleurotus florida* varied from 2.74 ± 0.13 mg/g DW to 3.34 ± 0.21 mg/g DW while in *Pleurotus ostreatus*, total tannin content ranged from 1.71 ± 0.46 mg/g DW to 2.93 ± 0.26 mg/g DW. The results indicated that the highest amount of total condensed tannin was found in ethanolic extract (3.97 ± 0.23 in *Pleurotus florida* and 3.31 ± 0.31 in

TABLE 4: EFFECT OF DIFFERENT SUBSTRATES ON THE ANTIOXIDANT CONTENTS OF PLEUROTUS FLORIDA AND PLEUROTUS OSTREATUS

Species	Substrates	Phenol (mg/g DW)		Flavonoid (mg/g DW)		Tannin (mg/g DW)	
		Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract
P. florida	A	1.57 ± 0.2	4.79 ± 0.18	2.50 ± 0.17	3.74 ± 0.05	2.74 ± 0.13	3.05 ± 0.14
	B	4.54 ± 0.12	7.64 ± 0.18	3.59 ± 0.11	4.4 ± 0.11	3.34 ± 0.21	3.97 ± 0.23
	C	2.64 ± 0.17	5.52 ± 0.21	3.49 ± 0.16	4.13 ± 0.11	3.26 ±0.17	3.70 ± 0.19
	D	3.99 ± 0.12	7.15 ± 0.23	2.38 ± 0.15	3.41 ± 0.18	2.99 ± 0.09	3.48 ± 0.24
P. ostreatus	A	2.20 ± 0.15	4.72 ± 0.24	1.69 ± 0.18	2.46 ± 0.24	1.71 ± 0.46	2.18 ± 0.3
	B	4.09 ± 0.17	6.88 ± 0.26	3.53 ± 0.42	4.40 ± 0.45	2.93 ± 0.26	3.31 ± 0.31
	C	2.59 ± 0.19	5.48 ± 0.2	3.14 ± 0.14	4.08 ± 0.11	1.85 ± 0.29	2.28 ± 0.37
	D	3.00 ± 0.31	5.98 ± 0.34	2.65 ± 0.17	3.64 ± 0.11	2.66 ± 0.25	3 ± 0.31

Pleurotus ostreatus) of both the species of *Pleurotus* cultivated on sugarcane bagasse Between the two species of *Pleurotus*, *Pleurotus florida* had the maximum amount of tannin than *Pleurotus ostreatus*. Among the substrates used for the present study, tannin was found to be maximum on sugarcane bagasse followed by banana waste in both the species than other substrates. **Arbaayah and Umi (2013)** stated that antioxidant properties in mushroom were mainly in the form of phenolic acids and flavonoids. Moreover, in mushrooms there were some main compounds with antioxidant effect **(Vamanu, 2014; Vangkapun *et al*., 2011).**

Vitamin C and Vitamin B_2

Mushrooms are one of the best sources of vitamins especially Vitamin B **(Mattila *et al*., 2000). Esselen and Fellers (1946)** gave a comprehensive data of vitamin content of mushrooms and some vegetable. Mushroom also contains vitamin C in small amounts (**Mattila *et al*., 2001**) and which are poor in vitamins A, D and E **(Anderson and Fellers, 1942).** The highest quantity of vitamin C and B_2 was obtained in the ethanolic extract of fruitbodies cultivated on sugarcane bagasse in both the species of *Pleurotus* **(Figure 5a & b)** while the lowest quantity of vitamin C and B2 was recorded (1.70 ± 0.03 mg/g DW; 1.4 mg/g ± 0.08) in the ethanolic extract of fruit bodies cultivated on paddy straw in *Pleurotus florida* and on banana waste, paddy straw in *Pleurotus ostreatus* (1.71 ± 0.05 mg/g DW; 1.4 ± 0.04). Between the solvents used for the present study, ethanolic extract showed the maximum amount of vitamins than the aqueous extract

TRACE ELEMENTS

Mushrooms are good source of minerals. Potassium, sodium, calcium and magnesium are considered to be the major mineral constituents while copper, zinc,

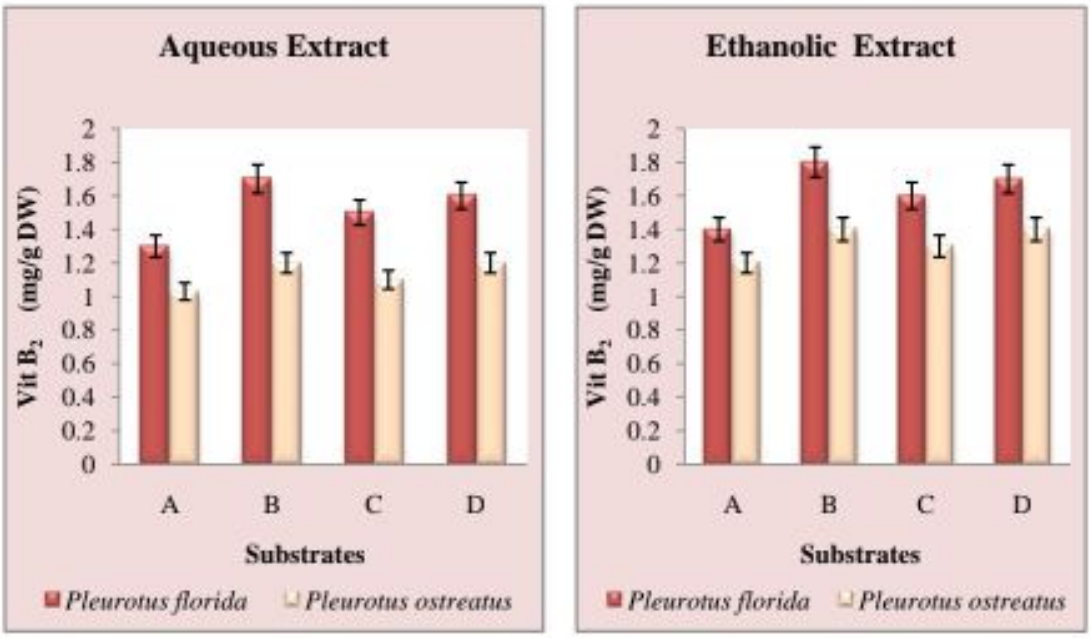


Figure 5a: Effect of different substrates on the Vitamin B_2 content of *Pleurotus florida* and *Pleurotus ostreatus*

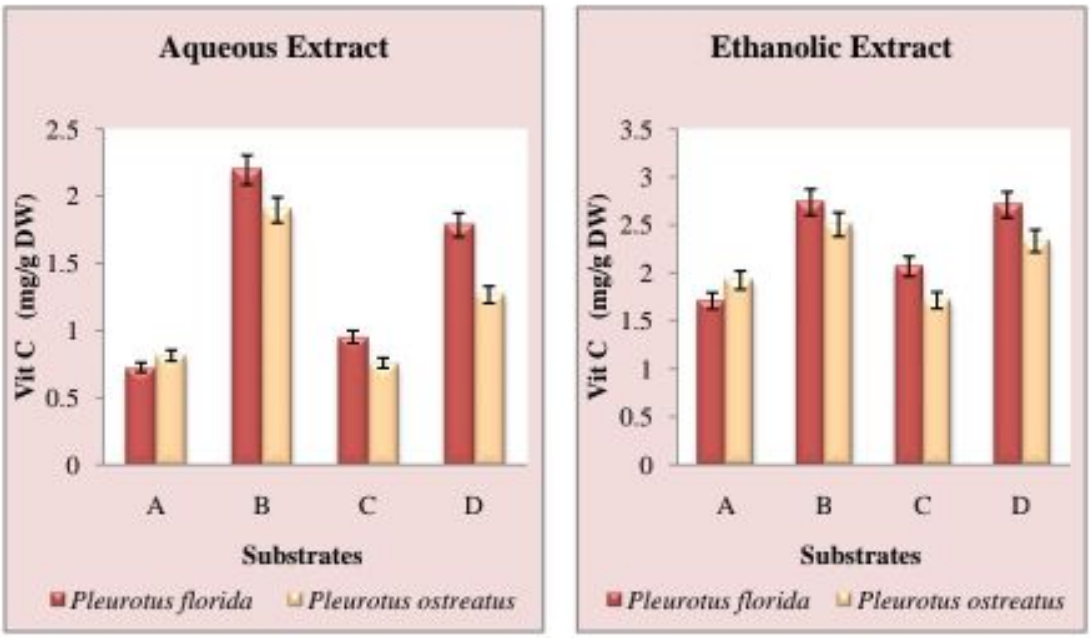


Figure 5b: Effect of different substrates on the Vitamin C content of *Pleurotus florida* and *Pleurotus ostreatus*

A – Paddy straw **B – Sugarcane bagasse**

C – Banana leaves **D – Substrate mixture**

iron and other trace elements such as manganese and cobalt make up the minor constituents. The amount of minerals in mushroom of the same species is directly related to factors such as species, growing area, growing time of fruiting body, genetic factors, substrates and distance from pollution sources **(Gucia *et al.*, 2012).** The amount trace elements present in two species of *Pleurotus* is shown in **Table – 5.** Values of 5 trace elements were determined in the selected mushroom species.

The copper content of the fruitbodies of *Pleurotus florida* and *Pleurotus ostreatus* was found to be 0.292 ppm and 0.342 ppm respectively. Copper is the third most abundant trace element in human body, with vitamin like impact on living systems. Small amount of copper is found in the human body but it plays a critical role in a variety of biochemical processes**.** Lead was totally absent in both the species of *Pleurotus*.

Among the five trace elements analyzed from the selected species, zinc was found to be higher in both the species of *Pleurotus* (2.721 ppm in *Pleurotus florida* and 3.311 ppm in *Pleurotus ostreatus* respectively). Mushrooms are said to be good biological accumulators of zinc and zinc is a biologically very vital to the human body **(Bano *et al.*, 1981).** Since these mushrooms are enough amount of zinc compared to other trace elements, their regular incorporation in the human diet will go a long way to overcome the retardation of growth and sexual maturity which has been linked with zinc deficiency **(Vallee, 1981).**

In the present study, it was recorded that the cadmium content was found to be minimum (0.014 ppm in *Pleurotus florida* and 0.019 in *Pleurotus ostreatus*) in both the species of *Pleurotus.* Cadmium is highly toxic metal. It was reported that cadmium is accumulated mainly in kidney, spleen and liver and its blood

TABLE 5: MICRONUTRIENT CONTENTS OF PLEUROTUS FLORIDA AND PLEUROTUS OSTREATUS CULTIVATED ON SUBSTRATE MIX

S. No.	Micronutrient	*Pleurotus florida* (in ppm)	*Pleurotus ostreatus* (in ppm)
1.	Copper	0.292	0.342
2.	Lead	-	-
3.	Zinc	2.721	3.311
4.	Cadmium	0.014	0.019
5.	Cobalt	0.049	0.026

serum level increases considerably following mushroom consumption **(Kalač and Svoboda, 2000).** Thus, cadmium seems to be the most deleterious among heavy metals in mushroom.

FT – IR ANALYSIS

The FT-IR spectrum was used to identify the functional group of the active compounds on the peak value in the region of infrared region, The FT-IR spectrum of *Pleurotus florida* and *Pleurotus ostreatus* was illustrated in **Figures 6 -7.** The outcome of FT-IR functional groups of both the species *Pleurotus* is presented in **Tables (6 -9).**

The FTIR recorded for *Pleurotus florida* cultivated on paddy straw shows the absorption bands with various intensities. From the values, it was identified that a strong peak was noted at 1040.11cm^{-1} which shows the CO-O-CO stretching. Also, C-H stretch was observed at 880cm^{-1}. Medium peaks were observed at various absorption bands which shows the presence of N-H stretching, C–H stretch, C=C stretching, C–Br stretching.

Pleurotus florida cultivated on sugarcane bagasse shows the absorption bands which corresponds to O-H stretching, C-H stretching, C=C stretching, C-H bending, CO-O-CO stretching, C-Br stretching.

FTIR recorded for *Pleurotus florida* cultivated on banana leaves shows the absorption bands at 3430 cm^{-1} which corresponds to N-H stretching, 2923.93 cm^{-1} confirms the C-H stretch. A strong band at 1628.80cm^{-1} shows C=C stretching, 1040.11cm^{-1} shows CO-O-CO stretching, 880.45 cm^{-1} shows C=C bending.

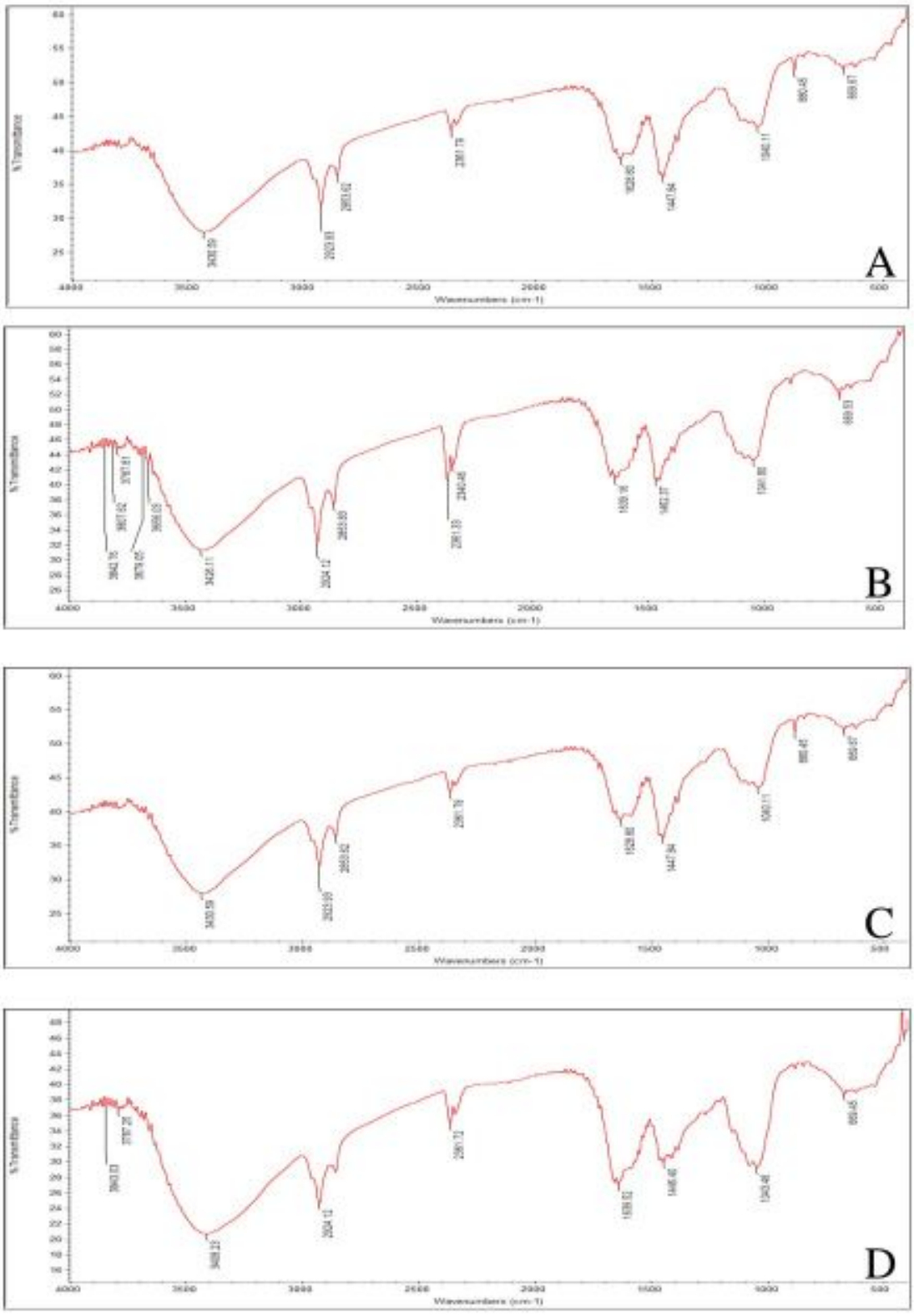


Figure 6: FT-IR spectrum of *Pleurotus florida* cultivated on different substrates

A – Paddy straw **B – Sugarcane bagasse**

C – Banana leaves **D – Substrate mixture**

TABLE 6 A: ASSIGNMENT OF INFRARED ABSORPTION BANDS FOR PLEUROTUS FLORIDA CULTIVATED ON PADDY STRAW

S. No.	Peak value	Functional group	Intensity
1.	3430.59	N-H stretching, aliphatic primary amine	Medium
2.	2923.93	C–H stretch, alkanes	Medium
3.	2853.62	C–H stretch, alkanes	Medium
4.	2361.79	H–C=O: C–H stretch, aldehydes	Medium
5.	1628.80	C=C stretching, α,β-unsaturated ketone	Strong
6.	1447.84	C-H bending, alkane	Medium
7.	1040.11	CO-O-CO stretching, anhydride	Strong, broad
8.	880.45	C–H "oop" aromatics	Strong
9.	669.67	C–Br stretch, alkyl halides	Medium

TABLE 6 B: ASSIGNMENT OF INFRARED ABSORPTION BANDS FOR PLEUROTUS FLORIDA CULTIVATED ON SUGARCANE BAGASSE

S. No.	Peak value	Functional group	Intensity
1.	3842.76	O-H stretching, alcohol	Medium, sharp
2.	3807.62	O-H stretching, alcohol	Medium, sharp
3.	3787.81	O-H stretching, alcohol	Medium, sharp
4.	3679.05	O-H stretching, alcohol	Medium, sharp
5.	3656.03	O-H stretching, alcohol	Medium, sharp
6.	3426.11	C-H stretching, alkyne	Strong, sharp
7.	2924.12	C-H stretching, alkane	Medium
8.	2853.83	C-H stretching, alkane	Medium
9.	2361.33	O=C=O stretching, carbon dioxide	Strong
10.	2340.46	O=C=O stretching, carbon dioxide	Strong
11.	1639.16	C=C stretching, alkene	Strong
12.	1462.37	C-H bending, alkane	Medium
13.	1041.80	CO-O-CO stretching, Anhydride	Strong, broad
14.	669.53	C-Br stretching, halo compound, Halo compound	Strong

TABLE 7A: ASSIGNMENT OF INFRARED ABSORPTION BANDS FOR PLEUROTUS FLORIDA CULTIVATED ON BANANA LEAVES

S. No.	Peak value	Functional group	Intensity
1.	3430.59	N-H stretching, aliphatic primary amine	Medium
2.	2923.93	C–H stretch, alkanes	Medium
3.	2853.62	C–H stretch, alkanes	Medium
4.	2361.79	H–C=O: C–H stretch, aldehydes	Medium
5.	1628.80	C=C stretching, α,β-unsaturated ketone	Strong
6.	1447.84	C-H bending, alkane	Medium
7.	1040.11	CO-O-CO stretching, anhydride	Strong, broad
8.	880.45	C=C bending, alkene	Strong
9.	669.67	C–Br stretch, alkyl halides	Medium

TABLE 7B : ASSIGNMENT OF INFRARED ABSORPTION BANDS FOR PLEUROTUS FLORIDA CULTIVATED ON SUBSTRATE MIX

S. No.	Peak value	Functional group	Intensity
1.	3843.03	O-H stretching, alcohol	Medium, sharp
2.	3787.25	O-H stretching, alcohol	Medium, sharp
3.	3408.23	N-H stretching, aliphatic primary amine	Medium
4.	2924.12	C–H stretch, alkanes	Medium
5.	2361.72	O=C=O stretching, carbon dioxide	Strong
6.	1639.52	C=C stretching, α,β-unsaturated ketone	Strong
7.	1446.40	C-H bending, alkane	Medium
8.	1043.46	CO-O-CO stretching, anhydride	Strong, broad
9.	669.45	C-Br stretching, halo compound, Halo compound	Strong

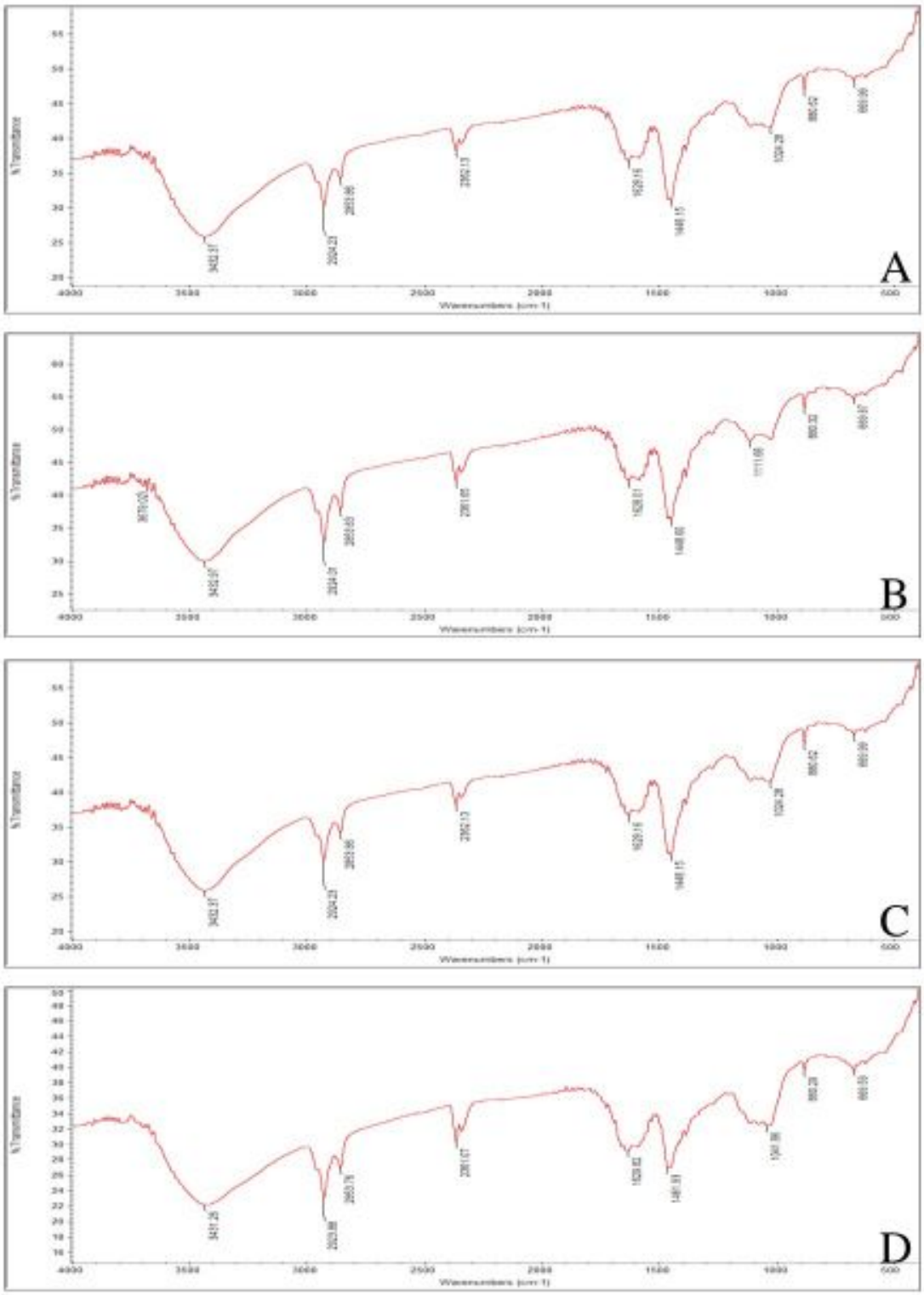


Figure 7: FT-IR spectrum of *Pleurotus ostreatus* cultivated on different substrates

A – Paddy straw **B – Sugarcane bagasse**

C – Banana leaves **D – Substrate mixture**

TABLE 8A: ASSIGNMENT OF INFRARED ABSORPTION BANDS FOR PLEUROTUS OSTREATUS CULTIVATED ON PADDY STRAW

S. No.	Peak value	Functional group	Intensity
1.	3432.37	N-H stretching, aliphatic primary amine	Medium
2.	2924.23	N-H stretching, amine salt	strong, broad
3.	2853.86	C-H stretching, alkane	Medium
4.	2362.13	O=C=O stretching, carbon dioxide	Strong
5.	1628.16	C=C stretching, α,β-unsaturated ketone	Strong
6.	1448.15	C-H bending, alkane	Medium
7.	1024.28	CO-O-CO stretching, anhydride	Strong, broad
8.	880.62	C=C bending, alkene	Strong
9.	669.99	C-Br stretching, halo compound, Halo compound	Strong

TABLE 8B: ASSIGNMENT OF INFRARED ABSORPTION BANDS FOR PLEUROTUS OSTREATUS CULTIVATED ON SUGARCANE BAGASSE

S. No.	Peak value	Functional group	Intensity
1.	3679.02	O-H stretching, alcohol	medium, sharp
2.	3432.97	O-H stretching, alcohol	strong, broad
3.	2924.01	N-H stretching, amine salt	strong, broad
4.	2853.63	C-H stretching, alkane	Medium
5.	2361.65	O=C=O stretching, carbon dioxide	Strong
6.	1628.01	C=C stretching, α,β-unsaturated ketone	Strong
7.	1448.60	C-H bending, alkane	Medium
8.	1111.68	C-O stretching, tertiary alcohol	Strong
9.	880.32	C=C bending, alkene	Strong
10.	669.67	C-Br stretching, halo compound, Halo compound	Strong

TABLE 9A: ASSIGNMENT OF INFRARED ABSORPTION BANDS FOR PLEUROTUS OSTREATUS CULTIVATED ON BANANA LEAVES

S. No.	Peak value	Functional group	Intensity
1.	3432.37	N-H stretching, aliphatic primary amine	Medium
2.	2924.23	N-H stretching, amine salt	strong, broad
3.	2853.86	C-H stretching, alkane	Medium
4.	2362.13	O=C=O stretching, carbon dioxide	Strong
5.	1628.16	C=C stretching, α,β-unsaturated ketone	Strong
6.	1448.15	C-H bending, alkane	Medium
7.	1024.28	CO-O-CO stretching, anhydride	Strong, broad
8.	880.62	C=C bending, alkene	Strong
9.	669.99	C-Br stretching, halo compound, Halo compound	Strong

TABLE 9B: ASSIGNMENT OF INFRARED ABSORPTION BANDS FOR PLEUROTUS OSTREATUS CULTIVATED ON SUBSTRATE MIX

S. No.	Peak value	Functional group	Intensity
1.	3431.26	N-H stretching, aliphatic primary amine	Medium
2.	2923.88	N-H stretching, amine salt	strong, broad
3.	2853.76	C-H stretching, alkane	Medium
4.	2361.07	O=C=O stretching, carbon dioxide	Strong
5.	1629.82	C=C stretching, α,β-unsaturated ketone	Strong
6.	1461.93	C-H bending, alkane	Medium
7.	1041.86	CO-O-CO stretching, anhydride	strong, broad
8.	880.29	C=C bending, alkene	Strong
9.	669.59	C-Br stretching, halo compound, Halo compound	Strong

A sharp band was observed for *Pleurotus florida* cultivated on substrate mix at 3843.03 cm^{-1} for N-H stretching, 2361.07cm^{-1} for O=C=O stretching, 1629.82 cm^{-1} for C=C stretching. A strong band observed at 880.29 cm^{-1} confirms C=C bending band.

Pleurotus ostreatus cultivated on paddy straw shows a strong peak at 2924.3 cm^{-1} corresponding to N-H stretching, 2362.13 cm^{-1}, O=C=O stretching 1628.16 cm^{-1} C=C stretching. Also, a halo peak was noted at 669.99 cm^{-1}

Pleurotus ostreatus cultivated on sugarcane bagasse shows the stretching frequencies of O-H at (3679.02cm^{-1}, 3432.97cm^{-1}). A strong band was observed for CO-O-CO (1024.28cm^{-1}), C=C bending (1024.28cm^{-1}) C-Br stretching (669.99cm^{-1}).

Pleurotus ostreatus cultivated on banana leaves shows absorption bands at various intensities. A strong peak was observed at 2362.13 cm^{-1}, 1628.16 cm^{-1} which corresponds to O=C=O stretching, C=C stretching respectively. Also, halo peaks were observed at 669.99 cm^{-1}.

FTIR recorded for *Pleurotus ostreatus* cultivated on substrate mix shows strong absorption bands at 2361.07 cm^{-1} for O=C=O stretching , 1629.82 cm^{-1} for C=C stretching, 1041.86 cm^{-1} for CO-O-CO stretching, 880.29 cm^{-1} for C=C bending and a halo peak at 669.59 cm^{-1}

GC-MS ANALYSIS

Gas Chromatography – Mass Spectrometry analysis (GC - MS) is a powerful tool for qualitative and quantitative analysis of various compounds present in natural products and the technique has been widely applied in medical, biological and food research **(Kaluzna and Czaplinska, 2007).** The extracts of *Pleurotus florida* and

Pleurotus ostreatus were subjected to GC-MS analysis. The active principles with their retention time, molecular formula, molecular weight, molecular structure and peak area are presented in **Table 10 -11.**

Sixteen compounds were identified in ethanol extracts of *Pleurotus florida* cultivated on the mix of paddy straw + sugarcane bagasse + banana waste by GC – MS analysis **(Figure 8).** This mushroom can be a good source of phytoconstituents like n-hexadecenoic acid, tridecanoic acid, tetra decanoic acid, octadecanoic acid, oleic acid, vaccenic acid, eicosaidiene, methyl tetra decanoic acid. Three compounds namely 9-octadecanoic acid, oleic acid, cis vaccenic acid, 6-octadecanoic acid were found to be major in this fraction with 47.72% and 22.14% peak area respectively **(Table 10).**

GC – MS chromatogram of the ethanol extract of *Pleurotus ostreatus* cultivated on the mix paddy straw + sugarcane bagasse + banana waste showed 23 peaks indicating the presence of 23 bioactive compounds. The most abundant compounds that were present in dry *Pleurotus ostreatus* mushroom extract were cis – vaccenic acid, 6 octadecenoic acid and octadecanoic acid with highest peak areas of 31.68% and 10.27% shown in **Figure 9.** Most of the identified compounds has been reported to possess interesting biological activities (Tables and). Octadecanoic acid **(Rahuman *et al.*, 2000)** and oleic acid **(Awa *et al.*, 2012)** possessed antimicrobial activities while hexadecenoic acid **(Aparna *et al.*, 2012),** trans 13 octadecanoic acid **(Krishnamoorthy and Subramanian, 2014)** showed anti-inflammatory and antioxidant activity.

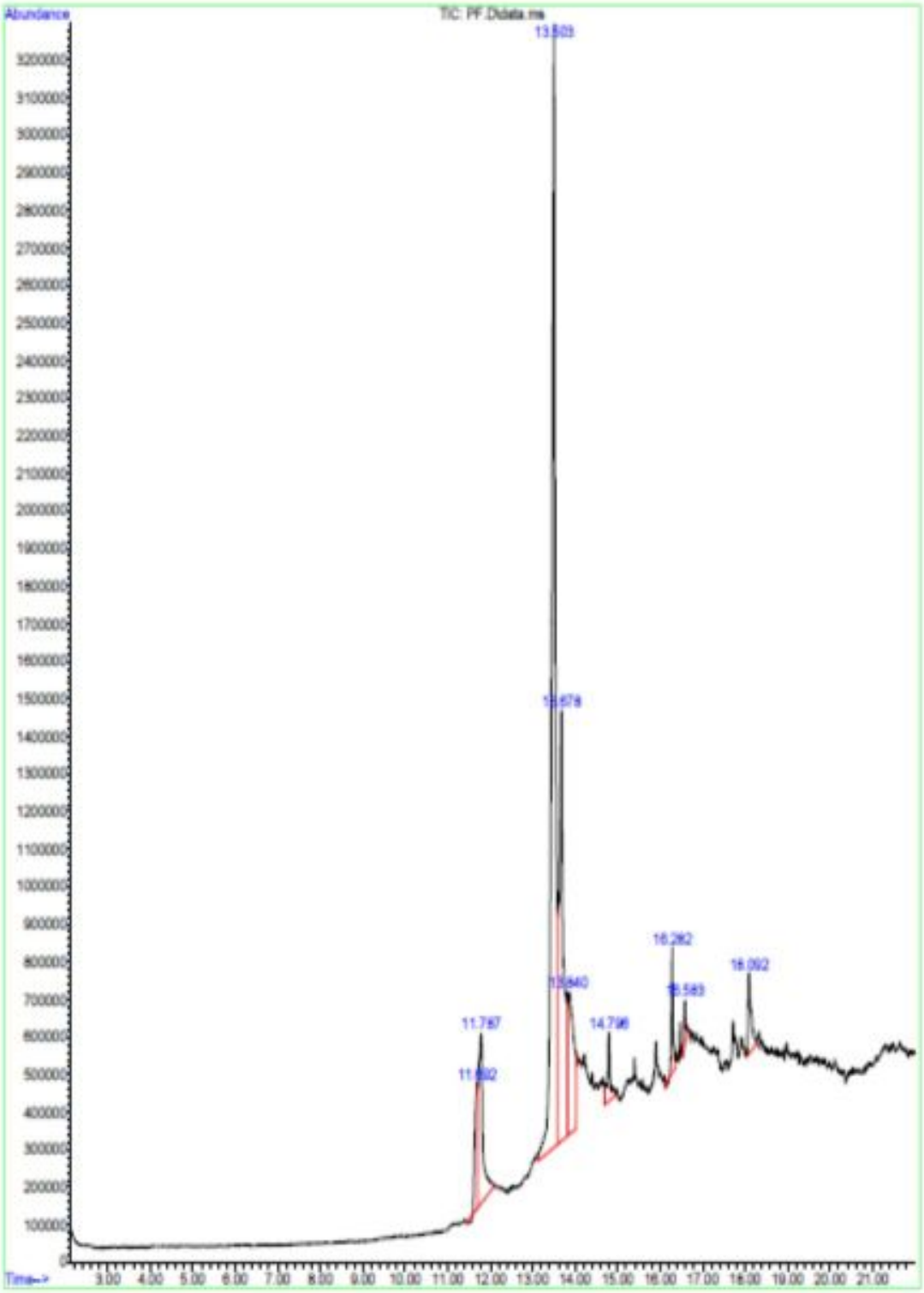
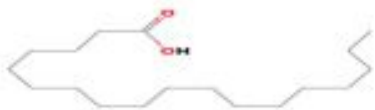
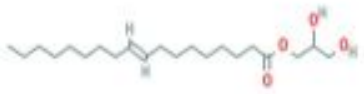


Figure 8: GC-MS spectrum of *Pleurotus florida* cultivated on substrate mix

TABLE 10: CHEMICAL CONSTITUENTS IDENTIFIED BY GC-MS ANALYSIS FROM PLEUROTUS FLORIDA CULTIVATED ON SUBSTRATE MIX

S. No.	R/T	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %	Molecular Structure	Reported Bioactivity
1.	11.693	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	3.66		Anti-inflammatory **(Aparna *et al.*, 2012)**, Antioxidant, Hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor **(Kumar *et al.*, 2010)** potent mosquito larvicide **(Rahuman *et al.*, 2000).**
2.	11.693	Tridecanoic acid	$C_{13}H_{26}O_2$	214.3443	3.66		Unknown
3.	11.693	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3709	3.66		Larvicidal and repellent activity **(Sivakumar *et al.*, 2011)**

4.	11.788	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	9.11		Antimicrobial activity (**Rahuman *et al.*, 2000**).
5.	13.499	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.4614	47.72		Antihypertensive, Increases HDL & decrease LDL (**Arora *et al.*, 2017**)
6.	13.499	Oleic Acid	$C_{18}H_{34}O_2$	282.4614	47.72		Antibacterial (**Awa *et al.*, 2012**)
7.	13.499	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.4614	47.72		Unknown
8.	13.679	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	22.14		Unknown
9.	13.840	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	9.94		Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, antileukotriene-D4, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge, flavor (**Krishnamoorthy and Subramaniam, 2014**)

10.	14.795	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356.5	2.28		Unknown
11.	14.795	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	2.28		Unknown
12.	14.795	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.5399	2.28		Unknown
13.	16.279	9-Methyl-Z-10-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268.4	2.02		Unknown
14.	16.279	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	2.02		Therapeutic uses in medicine, surgery (**Arora *et al.*, 2017**)
15.	16.279	1,19-Eicosadiene	$C_{20}H_{38}$	278.5157	2.02		Unknown
16.	16.582	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240.38	0.43		Unknown

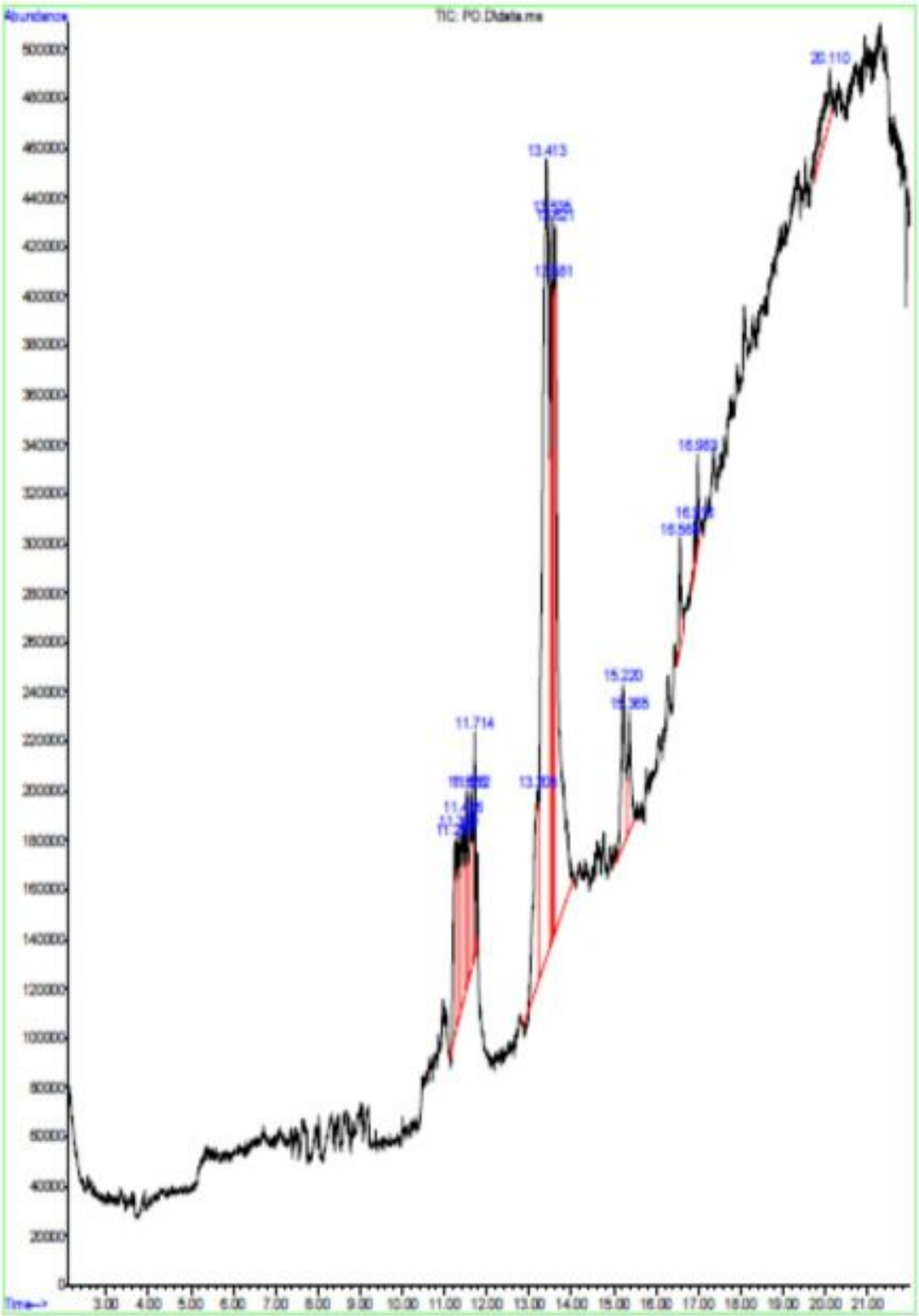
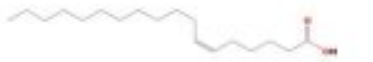
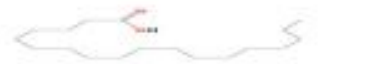
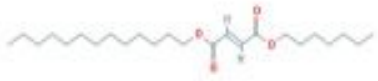
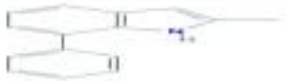


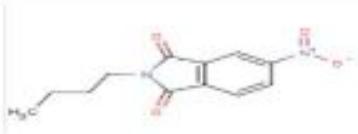
Figure 9: GC-MS spectrum of *Pleurotus ostreatus* cultivated on substrate mix

TABLE 11: CHEMICAL CONSTITUENTS IDENTIFIED BY GC-MS ANALYSIS FROM PLEUROTUS OSTREATUS CULTIVATED ON SUBSTRATE MIX

S. No.	R/T	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %	Molecular Structure	Reported Bioactivity
1.	11.249	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	3.13		Anti-inflammatory **(Aparna *et al.*, 2012)**, Antioxidant, Hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor **(Kumar *et al.*, 2010)** potent mosquito larvicide **(Rahuman *et al.*, 2000).**
2.	11.523	Tridecanoic acid	$C_{13}H_{26}O_2$	214.3443	2.71		Unknown
3.	13.206	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.4614	5.75		Unknown

4.	13.206	Oleic Acid	$C_{18}H_{34}O_2$	282.4614	5.75		Antibacterial **(Awa *et al.*, 2012)**
5.	13.414	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.4614	31.68		Unknown
6.	13.414	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	31.68		Unknown
7.	13.537	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	10.27		Antimicrobial activity **(Rahuman *et al.*, 2000).**
8.	15.220	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.5399	3.69		Unknown
9.	15.220	17-Pentatriacontene	$C_{35}H_{70}$	490.9303	3.69		Unknown
10.	15.220	Adamantane-1-(3,3-dichloropropyn...	$C_{13}H_{16}C_{l2}$	243.17	3.69		Unknown
11.	15.362	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.5	2.15		Unknown

12.	15.362	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282.4614	2.15		Unknown
13.	15.362	3-Eicosene, (E)-	$C_{20}H_{40}$	280.5316	2.15		Unknown
14.	16.563	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	207.2704	1.50		Unknown
15.	16.563	Fumaric acid, 4-heptyl tridecyl ester	$C_{24}H_{44}O_4$	396.6	1.50		Unknown
16.	16.563	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296.58	1.50		Unknown
17.	16.913	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{48}O_7Si_8$	577.2	0.32		Unknown
18.	16.913	2-Methyl-7-phenylindole	$C_{15}H_{13}N$	207.27	0.32		Unknown

19.	16.913	5-Methyl-2-phenylindolizine	$C_{15}H_{13}N$	207.27	0.32		Unknown
20.	16.979	Hexanoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester	$C_{16}H_{26}O_2$	250.38	0.83		Unknown
21.	16.979	1,2,5-Oxadiazol-3-amine, 4-(4-me...	$C_3H_5N_3O$	99.09	0.83		Unknown
22.	20.109	1H-Isoindole-1,3(2H)-dione,2-butyl-5-nitro-	$C_{12}H_{12}N_2O_4$	248.23468	2.57		Unknown
23.	20.109	Anthracene, 9,10-dihydro-9,9,10-trimethyl-	$C_{17}H_{18}$	222.32	2.57		Unknown

PHYSICO-CHEMICAL PROPERTIES OF THE SUBSTRATES

Substrates used in mushroom cultivation have effect on chemical, functional and sensorial characteristics of mushrooms **(Oyetayo and Ariyo, 2013).** *Pleurotus* species is a saprophyte and it extracts its nutrients from the substrates through its mycelium, obtaining substances necessary for its development such as carbon, nitrogen, vitamins and minerals **(Urben, 2004).** The physical properties of the substrates such as pH (**Figure 10a and 10b**), moisture content (water holding capacity) and chemical properties such as cellulose, lignin, nitrogen and carbon contents are presented in **Table 12.**

pH

Each mushroom has its optimal pH range for development and it is variable. For example, pH between 4 to 7 for the mycelium and 3.5 to 5.0 for the formation of basidiocarp **(Urben, 2004).** In the present study, it was recorded that the initial pH was higher in banana waste (6.9) followed by paddy straw (6.53) and the mix of paddy straw + sugarcane bagasse + banana waste (6.1) while pH was less in sugarcane bagasse (5.5) compared to other substrates **(Figure 10 a).** Our results agree with the study done by **Kalmis *et al.* (2008)** who reported that the optimum pH for mycelial growth and subsequent fruiting body development is obtained between the pH 6.5 to 7.0.After mushroom cultivation, pH of the spent substrate was also noted **(Figure 10b)**. pH of the spent substrates ranged from 4.76 to 5.84 in *Pleurotus florida* and 4.7 to 5.79 in *Pleurotus ostreatus.* With fungal colonization, the substrate pH is reduced for the reduction of organic acid.

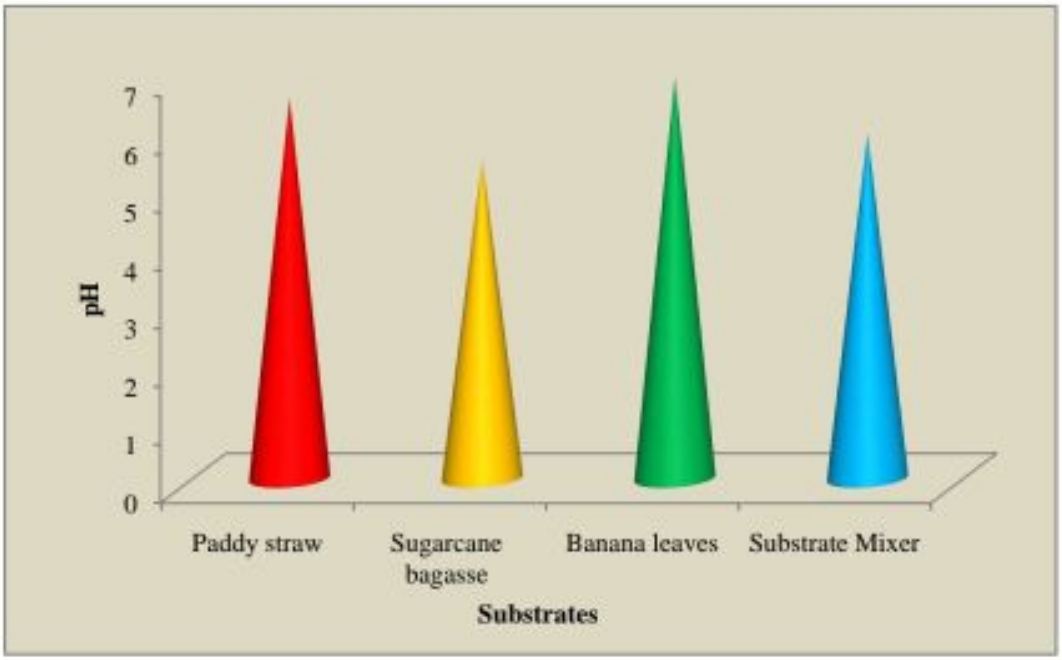


Figure 10a: pH of the substrates before the cultivation of *Pleurotus florida* and *Pleurotus ostreatus*

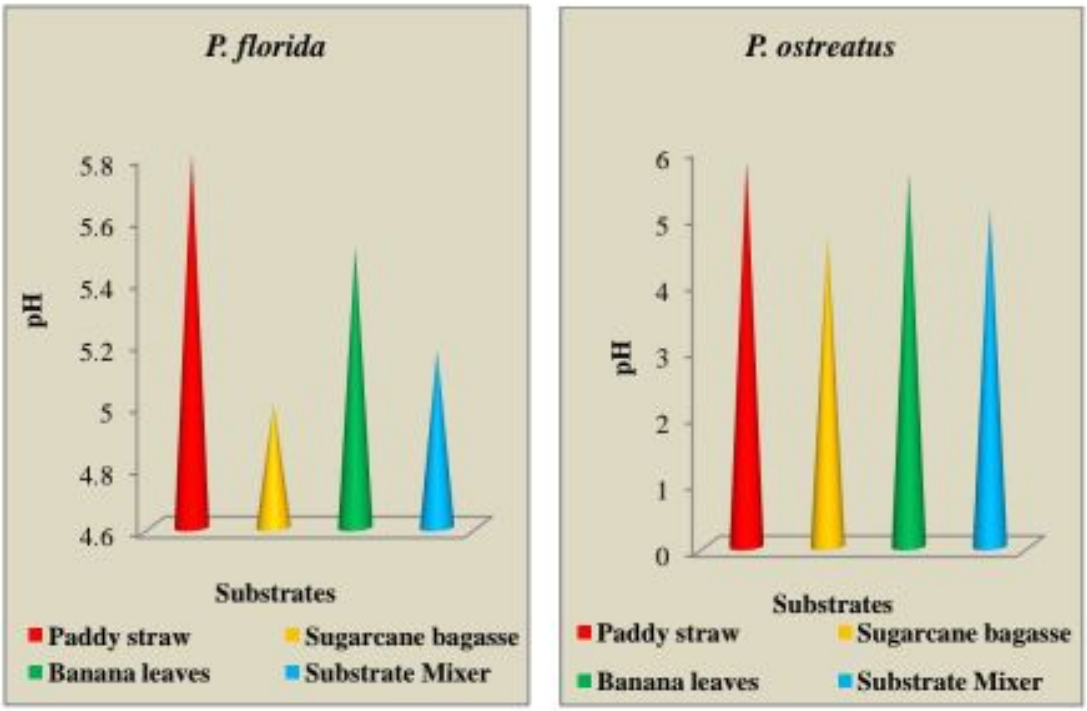


Figure 10b: pH of the substrates after the cultivation of *Pleurotus florida* and *Pleurotus ostreatus*

Moisture Content (Water Holding Capacity)

Water is one of the main factors that influence the success in mushroom growth. Nutrients are transported from the mycelium to the fruiting bodies by a steady moisture flow **(Oei and Nieuwenhuijzen, 2005).** In the present study, it was observed that water holding capacity was maximum in sugarcane bagasse (580.23 g) followed by paddy straw (385. 4 g) and banana waste (365.9 g) **(Table 12)**. High moisture content in the substrate will result in difficult breathing for the mycelium, inhibiting perspiration, rendering the development of fruiting body impossible even with elevated inoculum amounts or number of holes in mushroom cultivation packages, resulting in the development of non- desired organisms such as bacteria and nematodes **(Urben, 2004).**

In the present study, moisture content was maximum in sugarcane bagasse and it was noticed that the growth and development of fruit bodies on sugarcane bagasse was minimum and development of moulds was also observed on sugarcane bagasse. This may be due to the excess of moisture content in sugarcane bagasse. Our results agree with the study done by **Urben (2004).**Increasing moisture level is believed to reduce the porosity of the substrate, thus limiting oxygen transfer. For this reason, the use of high moisture content limited the growth within the whole substrate resulting in surface growth **(Patel *et al.*, 2009).**

Chemical Constituents of the Substrate

Table 5 shows the cellulose, lignin, carbon and nitrogen contents of different substrates used in the study. From the study, it was observed that the highest percentage of nitrogen and carbon was recorded banana waste (36.97 % C and 1.76 % N) followed by paddy straw (35.7% C and 1.68 N) and sugarcane bagasse

TABLE 12: PHYSICOCHEMICAL PROPERTIES OF THE SUBSTRATES USED FOR THE STUDY

Substrates	Water Holding Capacity		Lignin (mg/g)	Cellulose (mg/g)	Carbon (%)	Nitrogen (%)
	Initial Weight (g)	Final Weight (g)				
Paddy straw	100	385.4	21.1	178.05	35.7	1.68
Sugarcane bagasse		580.23	30.8	65.85	34.7	1.61
Banana leaves		365.9	25.7	331.71	36.97	1.76

(34.7% C and 1.61% N). Similarly, the cellulose content was found to be maximum in banana waste (331.71 mg/g DW) followed by paddy straw (178.05 mg/g DW) and sugarcane bagasse (65.85 mg/g DW). It was also noticed that sugarcane bagasse which had the highest lignin content (30.8%) and low cellulose and nitrogen content contents. The same substrate also showed the lowest yield compared to other substrates. Our results agree with the study done by **Badu *et al.* (2011)** who reported that this can be attributed to the fact that lignin is heterogenous and irregular arrangement of phenylpropanol polymer that resists chemical or enzymatic degradation to protect cellulose. The availability of the sugars to the fungi was limited by the lignin content.

ANTIOXIDANT ACTIVITY

Kettawan *et al.* (2011) and Selvi *et al.* (2007) have demonstrated that mushrooms contain antioxidant. In the present study, antioxidant activity of aqueous and ethanolic extracts of *Pleurotus ostreatus* and *Pleurotus florida* was examined using three different assays.

Ferric Ion Reducing Antioxidant Assay / Reducing Power

Figure 11a shows the ferric ion reducing antioxidant power assay of aqueous and ethanolic extracts of *Pleurotus florida* and *Pleurotus ostreatus*. Higher reducing power (0.053 ± 0.05 OD) was evident in ethanolic extract of *Pleurotus florida* cultivated on sugarcane bagasse. Between the solvents used for the present study, ethanolic extract showed the highest reducing power than the aqueous extract in both the species. Between the two species of *Pleurotus*, *Pleurotus ostreatus* showed the highest reducing power than *Pleurotus florida*. Among the substrates used for the present study, reducing power was high in *Pleurotus florida Pleurotus ostreatus* cultivated on sugarcane bagasse. Studies show that reducing power was directly

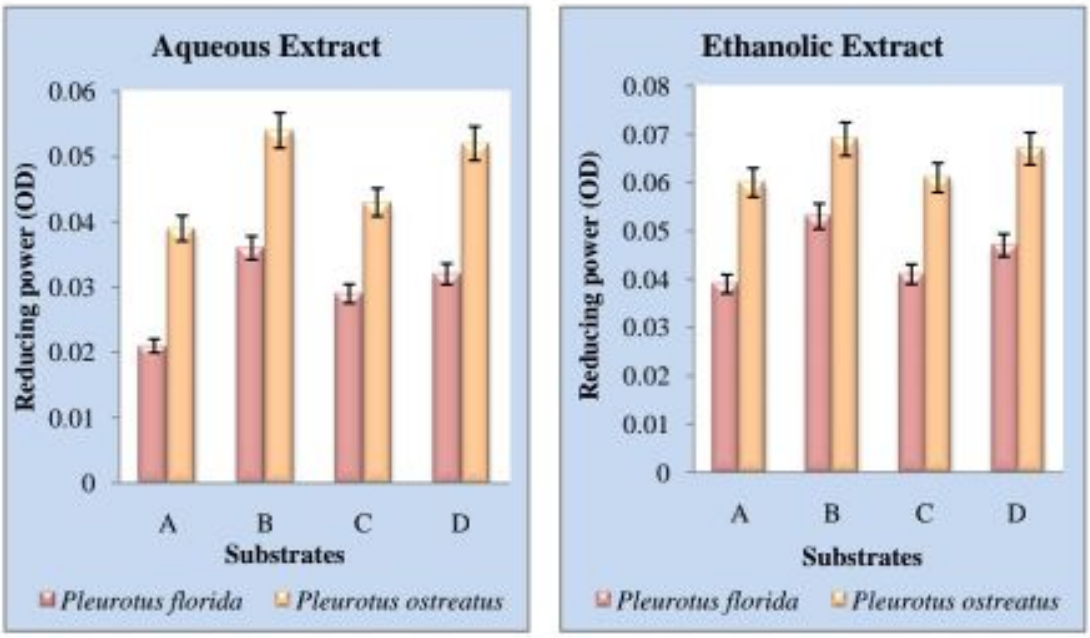


Figure 11a: Effect of different substrates on the ferric ion reducing antioxidant assay of *Pleurotus florida* and *Pleurotus ostreatus*

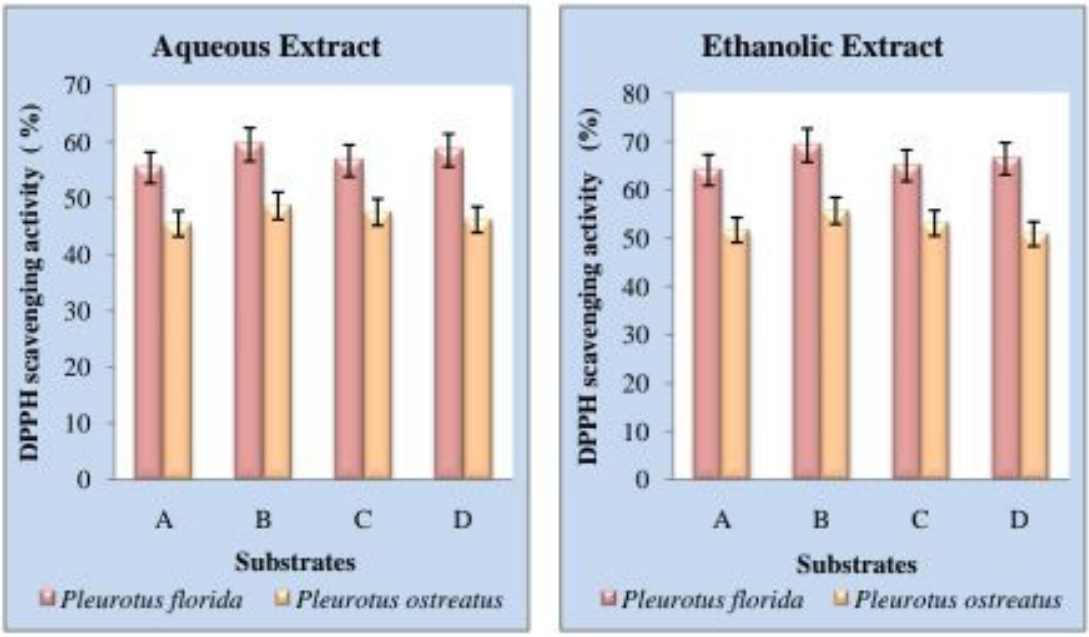


Figure 11b: Effect of different substrates on the DPPH assay of *Pleurotus florida* and *Pleurotus ostreatus*

A – Paddy straw **B – Sugarcane bagasse**

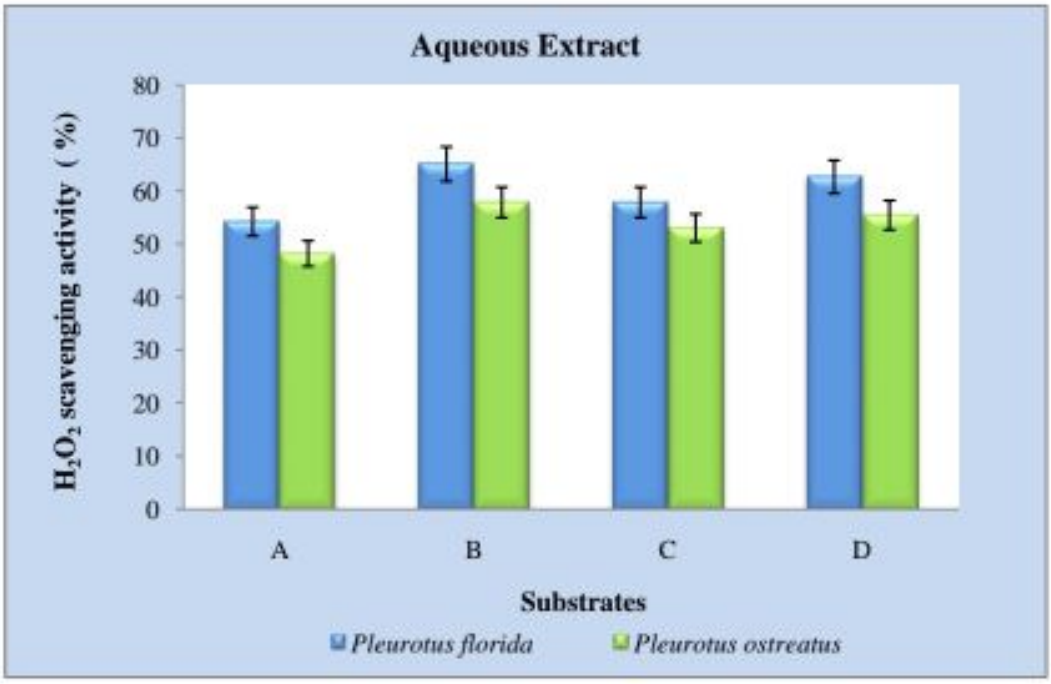
C – Banana leaves **D – Substrate mixture**

related to the antioxidant potential **(Amarowicz *et al.*, 2004).** Reducing power was generally linked to the reductones which by donating hydrogen break the chain reaction and exert antioxidant activity **(Gordon, 1990).** Reductones also prevent peroxides formation by reacting with precursors of peroxides and contribute to reducing power **(Duh *et al.*, 1999).**

Scavenging Effect on DPPH Free Radicals:

One of the most common methods for determination of antioxidant capacity is the DPPH free radical scavenging activity assay which relies on the reduction of methanolic DPPH solution in the presence of a hydrogen donating compound (antioxidant). The resulting decolourisation upon absorption of hydrogen from the antioxidant is stoichiometric with respect to the degree of reduction and the remaining DPPH, measured after a certain time, corresponding intensity to the radical scavenging activity of the antioxidant **(Blois, 1958).**

DPPH radical scavenging activity of aqueous and ethanolic extracts of *Pleurotus ostreatus* and *Pleurotus florida* is shown in **Figure 11b.** The present study revealed that the ethanolic extract of both the *Pleurotus* species cultivated on sugarcane bagasse possessed the highest percentage of scavenging activity (69.23 ± 1.9 in *Pleurotus florida* and 55.69 ± 2.1 in *Pleurotus ostreatus*) than aqueous extract. Our results agree with the study done by **Govindappa *et al.* (2011).** However, the findings were in contrast with the findings found by **Alnajar *et al.* (2012).** The difference in scavenging activity is species dependent. Besides, most of the scavenging activities were probably due to light sensitivity of the DPPH radical although with variation of extracts **(Masalu *et al.*, 2012).** Between the two species of *Pleurotus*, *Pleurotus florida* showed the highest percentage of scavenging activity



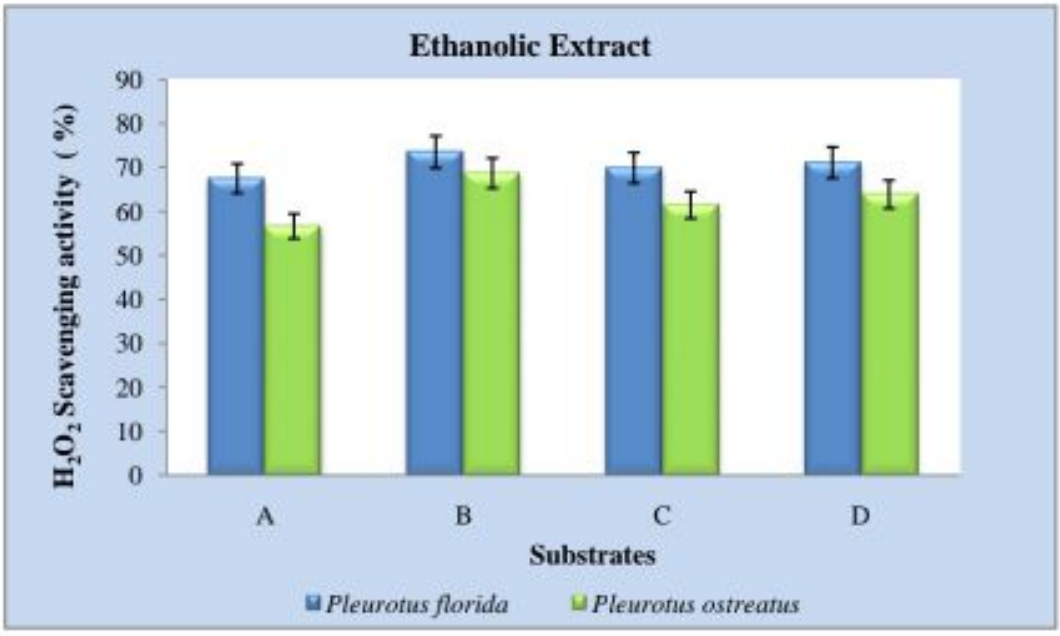


Figure 12: Effect of different substrates on the H_2O_2 assay of *Pleurotus florida* and *Pleurotus ostreatus*

A – Paddy straw **B – Sugarcane bagasse**

C – Banana leaves **D – Substrate mixture**

than *Pleurotus ostreatus*. From the present study, it was concluded that highest percentage of scavenging activity was noticed on sugarcane bagasse in both the species than other substrates.

Hydrogen Peroxide Scavenging Activity

In the present study, the hydrogen peroxide scavenging effect of aqueous and ethanolic extracts of *Pleurotus florida* and *Pleurotus ostreatus* is presented in **Figure 12.** The hydrogen peroxide scavenging activities of aqueous and ethanolic extracts of selected mushroom were in the order of sugarcane bagasse, mix of paddy straw + sugarcane bagasse + banana waste, banana waste alone and paddy straw alone. Scavenging effects of the mushroom extracts might be attributed to the presence of hydrogen donors such as phenolic compounds that could donate hydrogen from their hydroxyl groups to hydrogen peroxide converting it to water **(Yildiz *et al*., 2015).** Higher scavenging effect was observed for ethanolic extract of *Pleurotus* species and this might be connected to its higher total phenolic content in ethanolic extract than aqueous extract.

ANTIBACTERIAL ASSAY

The knowledge of drug has developed together with the evolution of scientific and social progress. Drugs derived from macro fungi are effective, easily available and less expensive and rarely have side effects. Initial screening for the potential antibacterial and antifungal compounds from mushroom may be performed by using the crude extracts. The most commonly used methods to determine antimicrobial susceptibility is the disc or agar well diffusion assay.

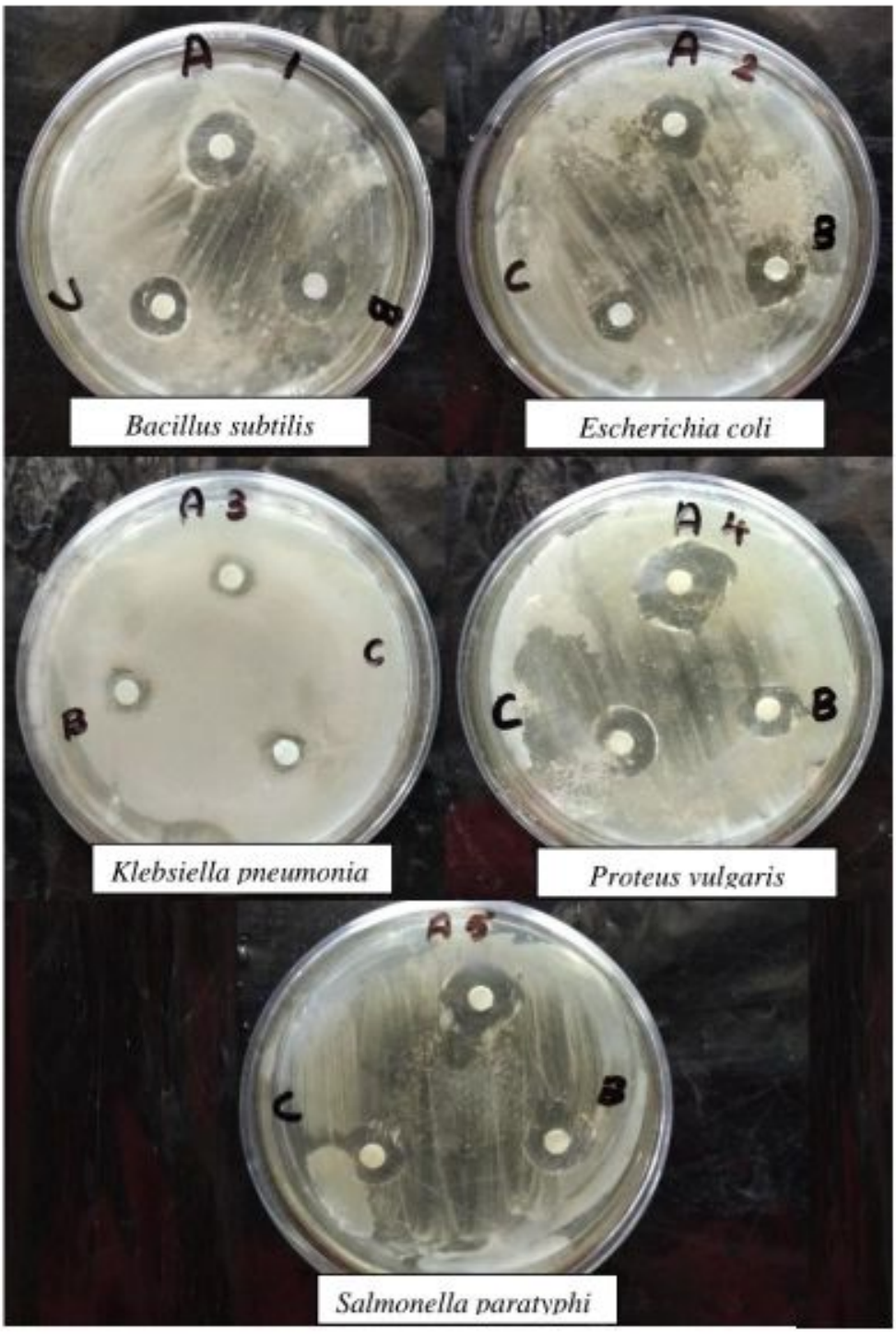
The antibacterial activity of *Pleurotus florida* and *Pleurotus ostreatus* ethanolic extracts was tested against five human bacterial pathogens such as *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris* and *Salmonella paratyphi* species. The specific zone of inhibition against different types of pathogenic bacteria is shown in **Table 13 and Plate 10.** Between the two species of *Pleurotus*, *Pleurotus florida* extract was found more effective against the selected bacteria than *Pleurotus ostreatus*. The maximum antibacterial activity of *Pleurotus florida* extract was found at 9 mm against *Bacillus subtilis* and minimum 3.5 mm against *Klebsiella pneumonia*. Similarly, the maximum antibacterial activity of *Pleurotus ostreatus* extract was found at 7.5 mm against *Proteus vulgaris* and minimum 2 mm against *Klebsiella pneumonia*. The ethanol extract of lyophilized mycelium of *Pleurotus ostreatus* had a strong antibacterial activity of some bacterial strains **(Wolff *et al.*, 2008).** *Pleurotus* species particularly *Pleurotus florida* had a narrow antibacterial spectrum against Gram-negative bacteria and strongly inhibited the growth of Gram-positive bacteria tested including *Bacillus subtilis* **(Loganathan *et al.*, 2008).**

SYNTHESIS OF SILVER NANOPARTICLES

Nanoparticles have a wide range of application as in combating microbes, biolabeling and in the treatment of cancer **(Travan *et al.*, 2009).** As mushroom extracts were mixed into aqueous solution of silver nitrate, it started to change the colour from watery to brown due to the reduction of silver ions, which indicated the formation of silver nanoparticles (**Plate 11).** UV – Vis spectroscopy is the significant method which gives the preliminary confirmation of silver nanoparticles. The fabricated silver nanoparticles showed absorption spectra ranging from 420 to 450 nm

Plate 10

ANTIBACTERAL ACTIVITY OF PLEUROTUS FLORIDA AND PLEUROTUS OSTREATUS CULTIVATED ON SUBSTRATE MIX



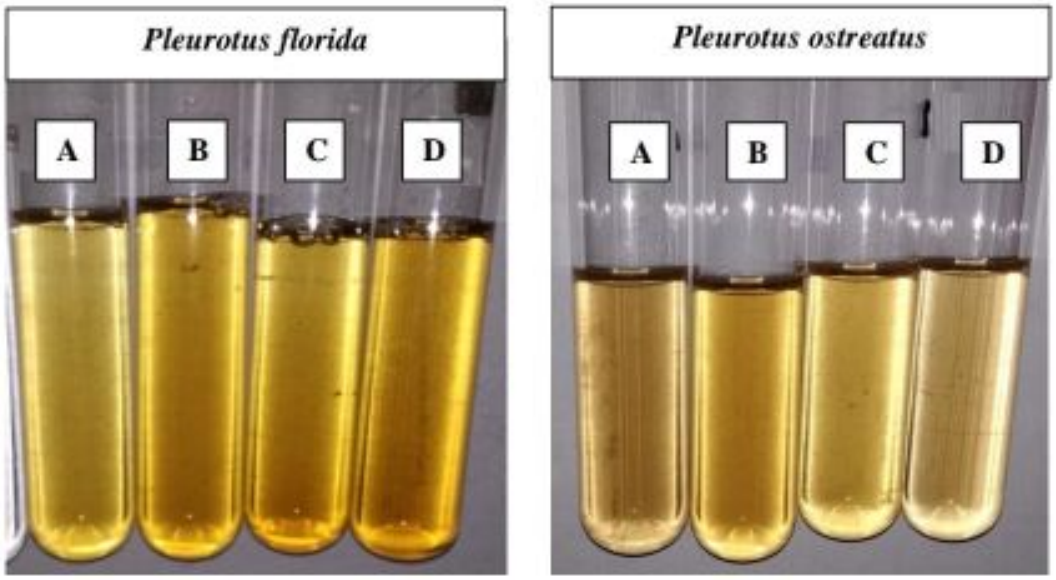
A – Control **B – *Pleurotus florida*** **C – *Pleurotus ostreatus***

TABLE 13: ANTIBACTERIAL ACTIVITY OF PLEUROTUS FLORIDA AND PLEUROTUS OSTREATUS CULTIVATED ON SUBSTRATE MIX

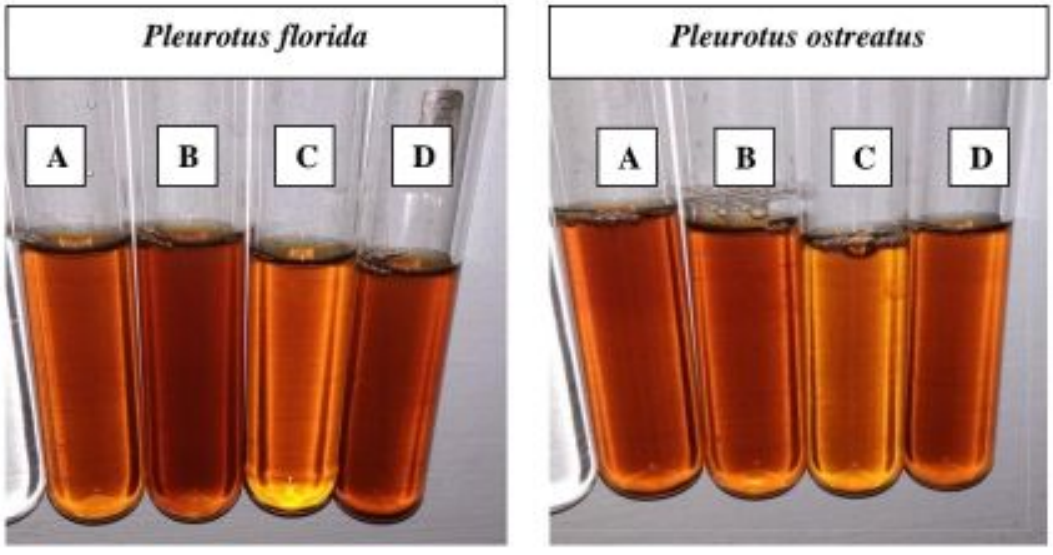
Bacteria	Zone of Inhibition (mm)		
	Positive control	*Pleurotus florida*	*Pleurotus ostreatus*
Bacillus subtilis	11	9	6.5
Escherichia coli	9	8	5.5
Klebsiella pneumonia	5	3.5	2
Proteus vulgaris	13	6	7.5
Salmonella paratyphi	9.5	7	5.5

Plate 11

VISUAL DETECTION OF SYNTHESIS OF SILVER NANOPARTICLES USING EXTRACTS OF PLEUROTUS FLORIDA AND PLEUROTUS OSTREATUS CULTIVATED UPON DIFFERENT SUBSTRATES



COLOUR CHANGE OF THE SOLUTION AFTER INCUBATING WITH $AgNO_3$ SOLUTION



A – Paddy Straw **B – Sugarcane bagasse**

C – Banana leaves **D – Substrate mix**

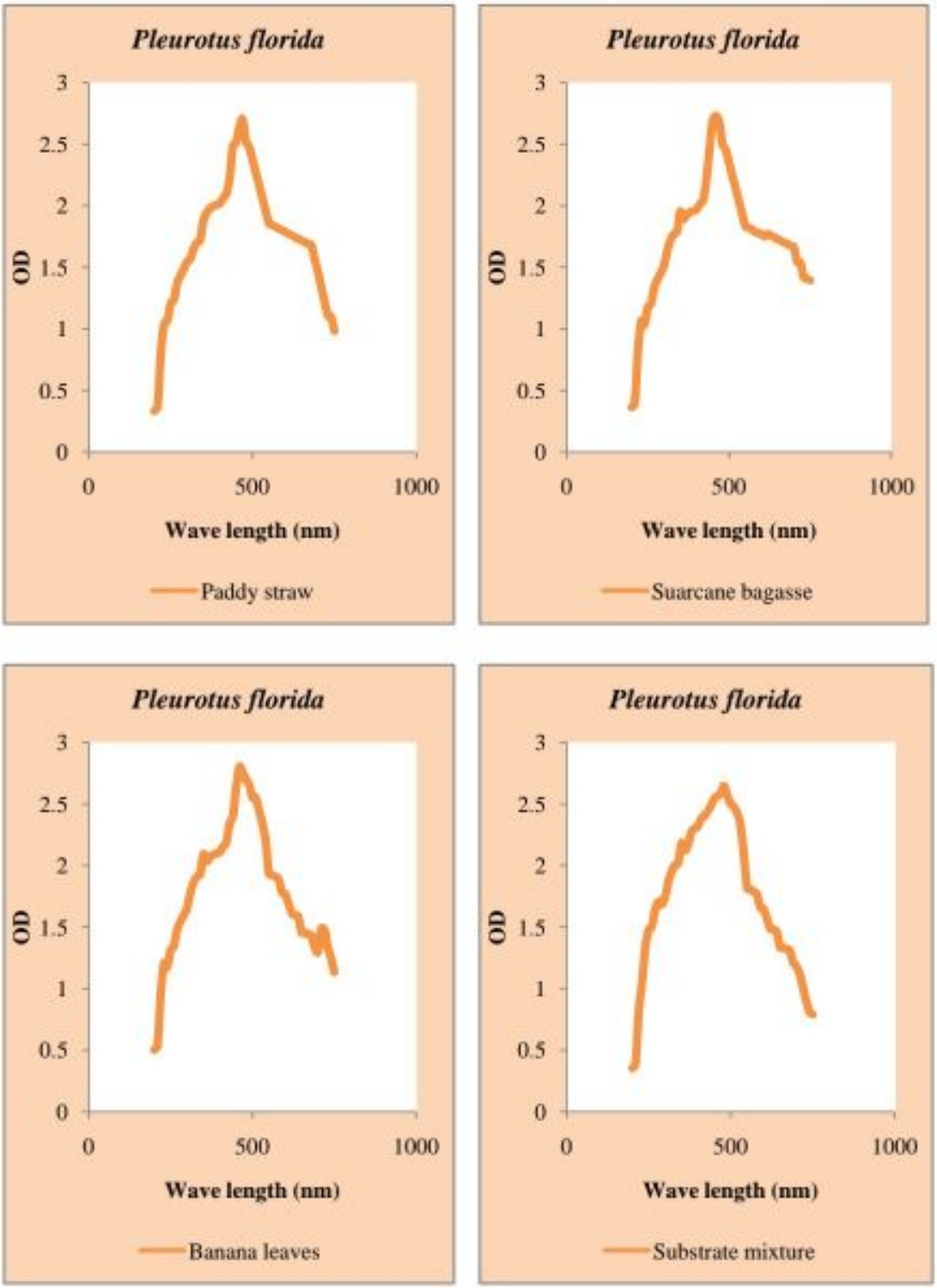


Figure 13: UV-Visible spectrum of silver nanoparticles synthesized by reduction of Ag^{+} ion solution with *Pleurotus florida* cultivated upon different substrates

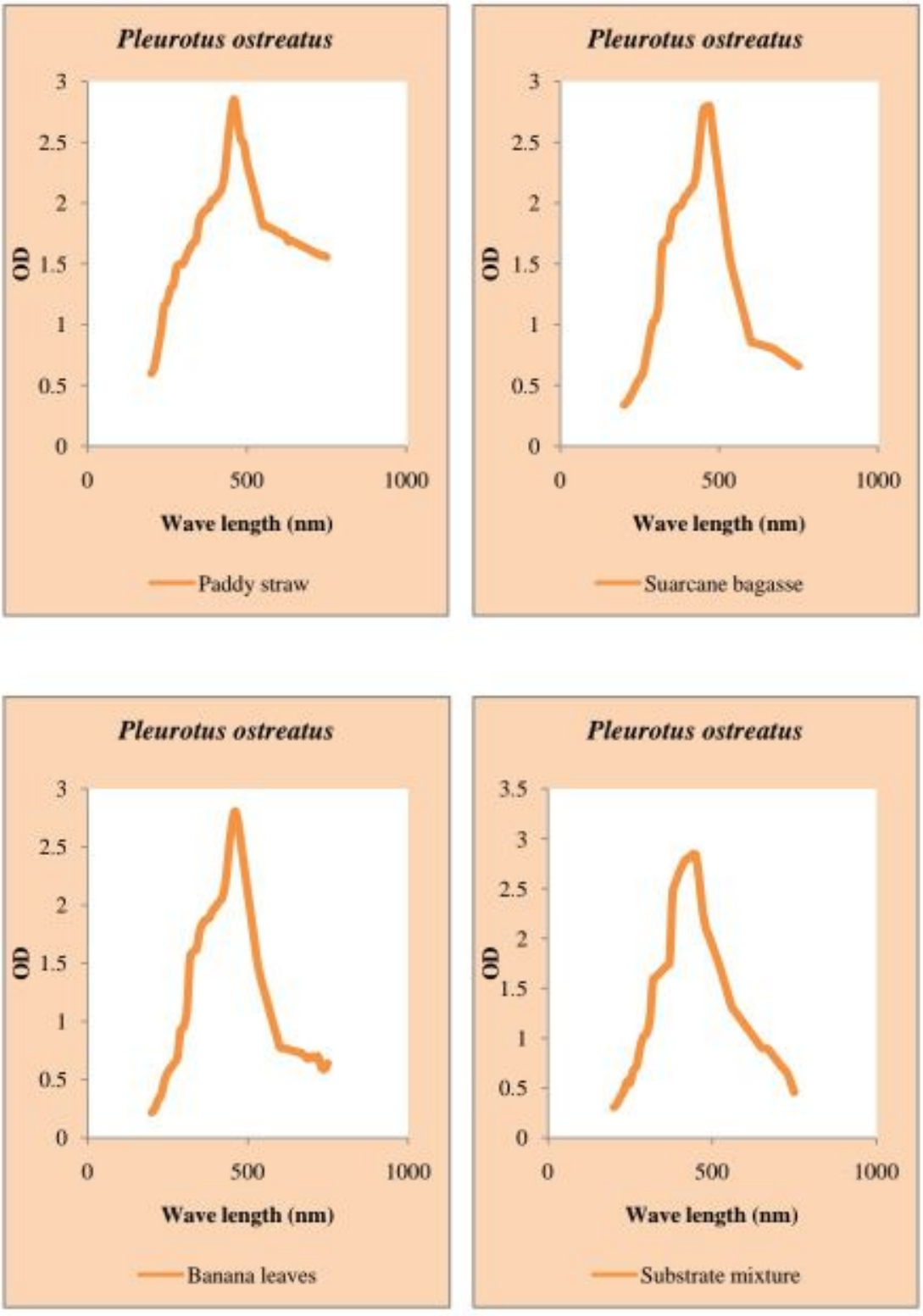


Figure 14: UV-Visible spectrum of silver nanoparticles synthesized by reduction of Ag^{+} ion solution with *Pleurotus ostreatus* cultivated upon different substrates

in both the extracts of *Pleurotus florida* and *Pleurotus ostreatus* **(Figure 13 -14).** Biologically synthesized silver nanoparticles could have many applications. Further studies are needed for synthesis and characterization of silver nanoparticles using mushroom extracts and also to explore the mechanism.

RECYCLING OF SPENT MUSHROOM SUBSTRATE

Mushroom growers are recycling spent mushroom substrate naturally and using it in agricultural and horticultural crops as manure at their own. Both mushroom growers and researchers have noticed that the application of SMS in soil enhances the crop yield and manages diseases in agricultural and horticultural crops. The present study was carried out to confirm the effect of SMS on germination and growth parameters on green gram. The results are presented in **Table 14.** The liquid fertilizer from SMS after 7 days of fermentation and used at dilution of 1:10. The results showed that the percentage of germination increased at 1% and 2% concentrations (97% and 90% respectively) than control (83%) while the germination percentage decreased at higher concentration (76.7% at 4% and 70% at 5%) than control (83%).

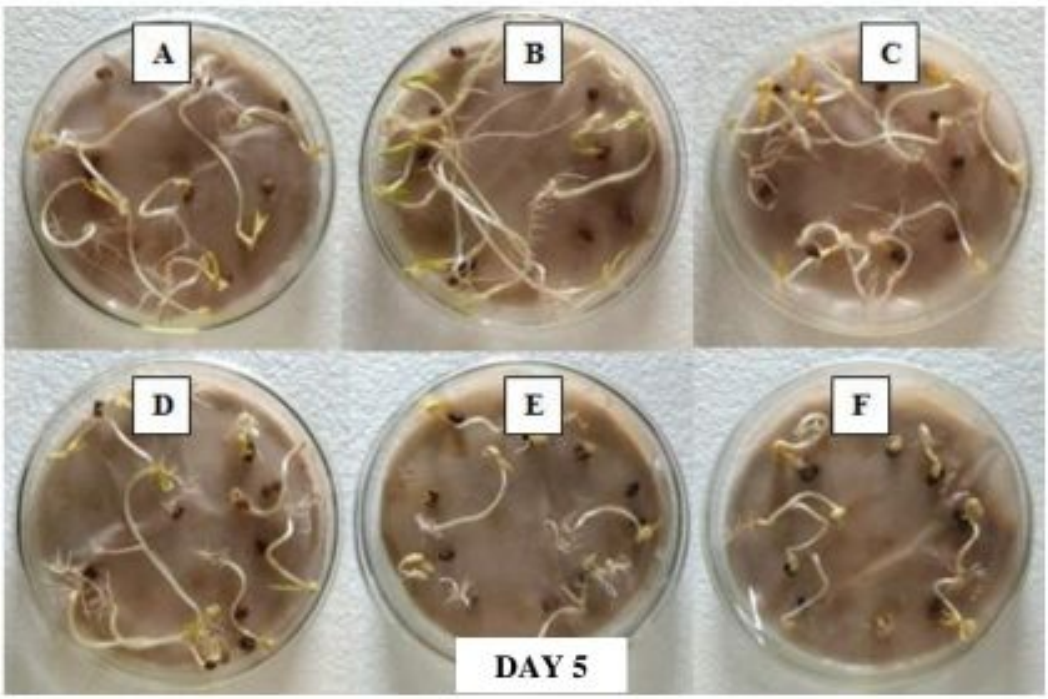
Growth parameters such as length of root and shoot were measured on 5^{th} and 10^{th} day **(Plate 12).** It was observed that root length and shoot length were found to be maximum only at 1% (13.7 ± 0.4 cm and 12.1 ± 1.9 cm on 5^{th} day; 15.4 ± 0.2 cm and 18.1 ± 1.4 cm on 10^{th} day respectively) than control (9.3 ± 1.8 cm and 9.6 ± 0.6 cm on 5^{th} day; 13.7 ± 2.1 cm and 15.9 ± 0.9 cm on 10^{th} day respectively). Spent mushroom substrate tended to produce better results than control. This could be due to the fact that during the process of composting / fermentation the nutrients are released into it **(Kadiri, 2002a and 2002b).** From the present study, it was concluded that spent

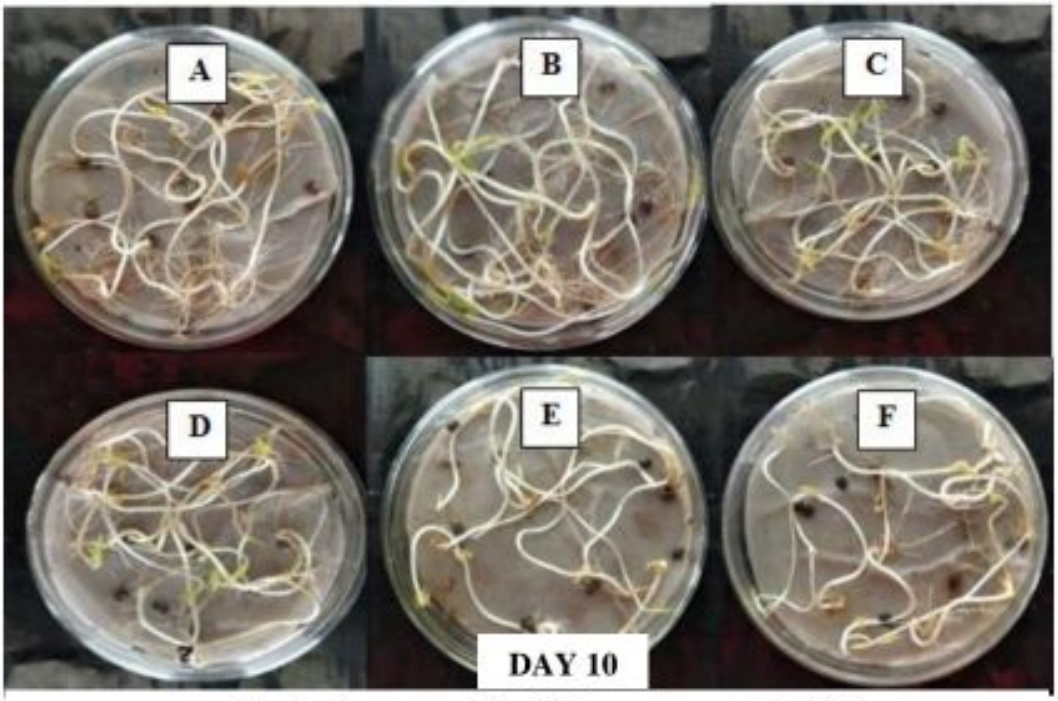
Table 14: EFFECT OF FERMENTED SMS LIQUID FERTILIZER ON THE GROWTH OF GREENGRAM

Treatment	Germination (%)	Day 5			Day 10		
		Root Length (cm)	Shoot Length (cm)	Vigour Index	Root Length (cm)	Shoot Length (cm)	Vigour Index
Control	83.3	9.3 ± 1.8	9.6 ± 0.6	1574.37	13.7± 2.1	15.9±0.9	2465.68
1%	96.7	13.7 ± 0.4	12.1 ± 1.9	2494.86	15.4 ± 0.2	18.1 ± 1.4	3239.45
2%	90	7.1 ± 1.3	9.2 ± 1.4	1467	13 ± 0.2	17.8 ± 0.3	2772
3%	83.3	6.8 ± 1.5	8.1 ± 0.15	1241.17	12.4 ± 0.3	13.1 ± 0.3	2124.15
4%	76.7	4 ± 1.1	5.4 ± 1.1	720.98	5.5 ± 0.4	10.7 ± 1	1242.54
5%	70	2.6 ± 1.1	5.9 ± 0.43	595	4.3 ± 0.2	9 ± 0.3	931

Plate 12

EFFECT OF SPENT MUSHROOM SUBSTRATE LIQUID FERTIIZER ON THE GROWTH OF GREENGRAM ON 5 AND 10 DAY OLD SEEDLING





A – Control **B – 1%** **C – 2%**

D – 3% **E – 4%** **F – 5%**

mushroom substrate, which presently has no economic value, is strongly recommended for use as a soil conditioner in order to enhance the yield of plants.

AWARENESS SURVEY

Mushrooms are found to be of highly nutritive value and a study of its cultivation from paddy straw and banana leaves and other agricultural waste is an eye opener for mass cultivation by common man. Hence a survey was taken from among farmers and students to analyze the use of mushroom and the understanding among the common people about its richness in food value.

The survey indicates that people lack awareness about its medicinal value and that it's an efficient means of conversion of agricultural waste into valuable protein and that it's a great source of additional income and employment. Based on the demand and supply ratio the marketing of mushroom usage should be encouraged by the nation as it is a good source of energy and strengthens a person as a means of livelihood.

The present work is carried on to give awareness to the people about the easy way of cultivation of mushroom and hence need to be popularised as a means of high food value rich in proteins and minerals. The coordinated efforts by the government will enhance mushroom cultivation which will also provide remunerative employment opportunities.

SUMMARY AND CONCLUSION

- Two species of *Pleurotus* namely *Pleurotus florida* and *Pleurotus ostreatus* were cultivated using three different substrates such as paddy straw, sugarcane bagasse, banana leaves and their mixture in 1:1:1 ratio.
- These three different substrates were investigated to determine the growth and yield of the selected species of *Pleurotus*. The following were observed in the study
    - *Pleurotus florida* showed the fastest colonization cultivated on paddy straw and maximum numbers of pin heads were observed in the same species on the same substrate.
    - In both the species, the percentage of fruitbodies developed from pin heads was maximum on banana waste.
    - *Pleurotus ostreatus* showed the maximum length and width of pileus on banana waste.
    - In both the species, yield of mushroom fruitbodies, biological efficiency and biomass loss were high on banana waste and paddy straw.
    - Growth parameters and yield were found to be low in both the species cultivated on sugarcane bagasse.
    - In the present study, the unknown specimen was observed in small clusters on mushroom packets filled with sugarcane bagasse.
- The result of preliminary phytochemical analysis showed that the extract obtained using ethanol possessed a greater number of constituents as compared to other solvents.

- Nutritional parameters were measured for both the *Pleurotus* species.
    - Moisture and ash contents were maximum in both the species of *Pleurotus* cultivated on sugarcane bagasse.
    - Carbohydrate, protein, lipid, vitamin C and vitamin B2 contents were found to be maximum in ethanolic extract of both the species of *Pleurotus* cultivated on sugarcane bagasse.
    - Amino acid content was found to be maximum in ethanolic extract of both the species of *Pleurotus* cultivated on the mix of paddy straw + sugarcane bagasse + banana waste.
- Antioxidant compounds were quantified and antioxidant activities ware studied for both the species of *Pleurotus*.
    - Phenol, flavonoid and tannin contents were found to be maximum in ethanolic extract of *Pleurotus florida* cultivated on sugarcane bagasse.
    - Highest percentage of scavenging activities such DPPH radical scavenging activity, hydrogen peroxide scavenging activity and reducing power was noticed in *Pleurotus florida* cultivated on sugarcane bagase and the mix of paddy straw + sugarcane bagasse + banana waste respectively.
- The antibacterial activity of *Pleurotus* species was tested against 5 human pathogens. *Pleurotus florida* extract was found more effective against the selected bacterial than *Pleurotus ostreatus*.
- Physico-chemical properties of the substrates were determined. The following observations were made.
    - The optimum pH for mycelial growth and subsequent fruiting body development is obtained between the pH 6.5 to 7.0.With fungal

colonization, the substrate pH is reduced for the reduction of organic acid.

- Moisture content was maximum in sugarcane bagase and it was noticed that the growth and development of fruitbodies on sugarcane bagase was minimum and development of moulds was also observed on sugarcane bagasse.
- It was observed that the highest percentage of nitrogen and carbon was recorded banana waste and cellulose content was found to be maximum in the same substrate.
- It was also noticed that sugarcane bagasse which had the highest lignin content (30.8%) and low cellulose and nitrogen content contents.

- The FT-IR analysis was done to identify the functional group of the active compounds on the peak value in the region of infrared region for both the species of *Pleurotus* cultivated on different substrates.
- 16 and 23 bioactive compounds were identified in ethanolic extracts of *Pleurotus florida* and *Pleurotus ostreatus* cultivated on themix of paddy straw + sugarcane bagasse + banana waste by GC – MS analysis.
- The amount of trace elements was determined and quantified for the selected mushroom species. Copper, zinc, cadmium and cobalt were very less in amount and lead was totally absent.
- Spent mushroom substrate were recycled and used as fertilizer for the growth of plants.

Edible mushrooms have been eaten and appreciated for their flavor, economic and ecological values and medicinal properties. They are able to grow under climatic conditions on cheap, readily available waste materials. These mushrooms are a clear

example of how low value waste which is produced primarily through the activities of the agricultural, forest and food processing industries, can be converted to higher value material useful to mankind. For many reasons, the fungus *Pleurotus* genus had been intensely studied and cultivated in many different parts of the world. This mushroom demands environmental controls for cultivation and its fruiting bodies are not often attacked by diseases and pest and it can be cultivated in a simple and cheap way. Another advantage of growing oyster mushroom is that a high percentage of the substrate is converted to fruiting bodies, increasing profitability as compared to other mushrooms making *Pleurotus* an excellent choice for mushroom cultivation.

1. The results of the present study support the efficient production of mushroom on substrates.
2. Utilizing these waste products as substrates for the production of mushrooms would reduce the adverse environmental effects of theses waste products.
3. An economical strategy for converting waste products into nutritious food source is represented in this study

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