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## Advances in applied mycology and fungal biotechnology\*

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### ABSTRACT

Fungi are achlorophyllous and eukaryotic living organisms specifically having chitinous cell wall and absorptive nutrition. The peculiar characters possessed by fungi made them to raise it to a level of Kingdom Mycota. Around 1.5 million fungi are estimated. One lakh species are reported from the world, while 29,000 fungal species are reported from India. Fungal world is so large that what we know is a drop of Atlantic Ocean. Nature represents a formidable pool of bioactive compounds and is more than ever a strategic source for new and successful commercial products. Fungi are well recognized to produce a wide variety of chemical structures, several of which are most valuable pharmaceuticals, agrochemicals and industrial products. The world of fungi provides a fascinating and almost endless source of biological diversity, which is a rich source for exploitation. Fungi form nature's hidden wealth and need exhaustive and in depth studies. In view of the above, studies on biodiversity and bioprospecting of fungi has gained momentum and importance. This review is a humble contribution pertaining to beautiful world of fungi and their biotechnological aspects.

**Keywords:** Agrochemicals, biodiversity, biotechnology, fungi, industrial products, pharmaceuticals.

### INTRODUCTION

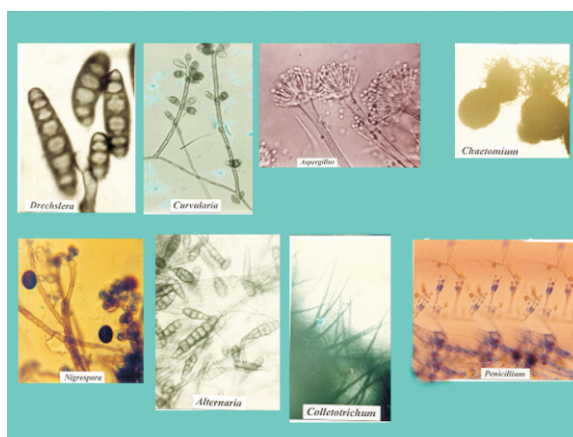
Fungi are known to colonize, multiply and survive in diversified habitats, viz. water, soil, air, litter, dung, foam, etc. Fungi are ubiquitous and cosmopolitan in distribution covering tropics to poles and mountain tops to the deep oceans. The kingdom of fungi contains 1.5 million fungal species, of which one lakh species are named. Many of the described species are known only as dead herbarium material and around 5-7% of species are isolated as pure cultures. Geographic location, climatic conditions, micro-habitat, substrate type and other factors influence the distribution of fungi around the world. Fungal flora of the United Kingdom, Korea, Cuba and other countries has been well explored for fungal species. Unlike prokaryotes, automated isolation techniques for fungi are not yet possible because of the extreme diversity in fungal distribution. Mapping of fungi is a challenging task due to the lack of sufficient taxonomic knowledge and shortage of mycologists around the world (Manoharachary *et al.*, 2005).

Fungi are non-chlorophyllous, holocarpic/eucarpic, microscopic or macroscopic living organisms which live as saprophytes and phytopathogens. The absence of chlorophyll has enforced them to live as parasites thus resulting in the causation of late blight of potato by *Phytophthora infestans*, wheat rust by *Puccinia graminis*, powdery mildew of wheat by *Erysiphe graminis*, corn smut with destructive effects by *Ustilago zaeae*, etc. The crop losses affect the economy and food security of a country and the world at large. Annual losses of crops due to devastating pathogens have brought famines like Irish famine, Bengal famine and others. However, the beneficial effects of fungi have been more promising and over shadowed the negative impacts of fungi. The present contribution of biotechnology to the overall world economy has been estimated to be in excess of US \$ 15 billion and of which 1/3 has been contributed by *Aspergillus*, *Penicillium* and *Cephalosporium*. The

biotechnological aspects and prospects of fungi are elaborated below. Taxonomically filamentous fungi comprise a heterogeneous collection of micro- and macro habitats from the heterotrophic saprophytic through symbiotic associations, as in mycorrhizas and lichens, to parasites (Manoharachary *et al.*, 2010).

Nature represents a formidable pool of bioactive compounds and is more than ever a strategic source for new and successful commercial products. Recent advances made in genomics, proteomics and combinatorial chemistry show that nature maintains compounds that have already the essence of bioactivity or function within the host and in the environment. Microbial sources such as fungi are well recognized to produce a wide variety of chemical structures, several of which are most valuable pharmaceuticals, agrochemicals and industrial products. The world of fungi provides a fascinating and almost endless source of biological diversity, which is a rich source for exploitation (**Fig. 1**).

Application of fungi and their sub cellular components, their metabolic processes and also their final products



**Fig. 1.** Diversity of fungi.

\* This research contribution is dedicated to the memory of late Dr. M. J. Thirumalachar, Eminent Mycologist, Biotechnologist and Microbiologist.

useful in industry, medicine, agriculture, environment management, food processing and other technologies for human welfare have been considered as fungal biotechnology. The fungal biotechnology aspect can further be divided into the production of useful end products such as baker's yeast, ethanol production, production of vitamins, enzymes, organic acids, single cell proteins, alkaloids, antibiotics, bioremediation, coal solubilization, ripening of cheese, paper industry, waste recycling, fermentation and other such activities. Since times immemorial fungi have been used by man and exploited for his benefit. Yeast has been used by man to make bread and alcohol for thousands of years, cultivated edible mushrooms, and fungi were also used in the fermentation, food processing and preparation of oriental foods like t mp , shoyu, miso and angko (Manoharachary *et al.*, 2010).

## FUNGAL ENZYMES

Use of enzymes forms an integral part of a wide variety of commercial processes and applications ranging from their use in the complex large scale manufacture of chemicals and processed foods to their simple inclusion in many commercial/ market products. Enzymes are now manufactured using diversified substrates, processes, fermentations and having specific activities. Fungi are known to produce large amounts of secretive enzymes, proteins and carbohydrate components. Fungal enzymes have widest variety of applications. Godfrey and Reichelt (1983) listed 260 sources of commercial enzymes and of which 27 are manufactured throughout the world. *Aspergillus* alone provides greatest variety of enzymes and *Aspergillus niger* alone produces 39 enzymes. Amylase, catalase, cellulase, chitinase, pectinase, glucanase, laccase, lipase, peptidase, tannase, tryptophan, xylanase and others are some of the enzymes produced by fungi (Table 1). *Acremonium chrysogenum*, *Alternaria tenuis*, *Aspergillus oryzae*, *Conidiobolus* sp. and *Coprinus cinereus* are some of the fungi which produce protease and urate oxidase enzyme is produced by *Absidia glauca*, *Aspergillus* sp., *Mucor* sp., *Fusarium* sp., *Penicillium* sp., etc. Commercial production of enzymes is now a major

business. Many thermotolerant enzymes such as cellulases and amylases have been isolated from thermophiles *Talaromyces emersonii* and xylanases from *Thermoascus aurantiacus*.

Industrial enzyme production is now recognized as a productive market and possibly leading to an excess of production. Future stress has to be on lowering the costs besides optimizing the fermentation and recovery process. Genetic engineering need to be applied to improve conventional enzymes.

Thermophilic fungi are economically very important and understanding of their genetics is essential. Gene amplification has opened new vistas in the microbial production of the desired products. In the field of biotechnology immobilized systems have already found wide-range of application, since they bypass many of the short-comings. In view of the advancements of technology there is a need for greater efforts to apply cell and enzyme immobilization technology to thermophilic fungal systems.

## LIGNIN DEGRADATION

Lignin is the widely distributed aromatic polymer which is renewable and is one of the major components of woody tissues besides it is 20-30% of the dry mass in woody plants. Wood is attacked by fungi and these wood rotting fungi are classified into three major types based on the type of wood decay caused by them, viz. white rot, brown rot and soft rot fungi. *Phanerochaete chrysosporium* has become the most commonly used model organism in lignin biodegradation studies. Lignases, lignin peroxidases, laccases, xylanases and other such enzymes are of paramount importance in biotechnological applications. The role of white rot fungi has been demonstrated in biopulping. The pulp and paper industry generates over 700 million or more gallons of toxic and coloured effluents which are treated with lignin decomposing fungi such as *P. Chrysosporium*.

## FUNGAL POLYSACCHARIDES

Among fungal polysaccharides, pullulan and scleroglucan (schizophyllan) and complex  $\beta$ -D- glucan are important. The first two are secreted by the cell and are soluble. These three are useful in industry and have market potential in commercial sector and medicine. Pullulan was first isolated by Bender *et al.* (1959) from *Aureobasidium pullulans*. It is used as a substrate for the assay of  $\alpha$ -1, 6- glucanase. The fungus also contains the melanin pigment and it offers protection against UV radiation, heavy metal toxicity and enzymatic attack. Scleroglucan is a neutral polysaccharide with a linear chain of  $\beta$ -1,3- linked D- glucosyl residues with a single  $\beta$ -1,3-linked D- glucosyl residue attached to every third sugar of the backbone. It is secreted by *Sclerotium glaucanicum*, *S. rolfsii* and *Schizophyllum commune*. This polysaccharide exhibits antitumor activity along with radiography and has shown significant reduction in cervical cancer. Schizophyllan has got various immunological properties that modify biological responses. The insoluble residue of the yeast is the  $\beta$ -D- glucan and allows its use in foods and medical therapy for the encapsulation and controlled release of flavor and drug because of its low toxicity.

**Table 1:** Some industrial enzymes produced by fungi

Enzyme	Fungus
$\alpha$ - amylase, lactase, melibase	<i>Aspergillus niger</i>
D- amylase	<i>Aspergillus niger</i>
Catalase	<i>Aspergillus niger</i>
Cellulase	<i>Chaetomium globosum</i> , <i>Trichoderma asperellum</i>
Dextranase	<i>Pencillium</i> sp.
$\alpha$ - glucanase	<i>Aspergillus oryzae</i> , <i>Aspergillus niger</i>
Hemicellulase	<i>Pencillium</i> sp.
Lipase	<i>Rhizopus arrhizus</i>
Pectinase	<i>Aspergillus niger</i>
Protease	<i>Aspergillus oryzae</i>
Rennet	<i>Mucor micheli</i>
Tannase	<i>Aspergillus niger</i>
Xylanase	<i>Aspergillus niger</i>

**Table 2:** Amino acids from fungi

Amino acid	Fungus
Tryptophan	<i>Hansenula anomala</i>
Lysine	<i>Candida pelliculosa</i> , <i>Saccharomyces lipolytica</i>
Methionine	<i>Saccharomyces lipolytica</i>
Glutamate	<i>Aspergillus oryzae</i>
Alanase	<i>Ustilago maydis</i>
Threonine	<i>Candida guilliermondii</i>
Phenylalanine	<i>Torula</i> sp.

## AMINO ACIDS FROM FUNGI

Fungi like all other microorganisms contain all of the amino acids, besides their occurrence in proteins and peptides in free and as soluble pools. Bacteria are considered as potential organisms as they produce amino acids in a shorter time. However, the filamentous fungi are superior in their ability to grow on wood wastes or other cheap materials. In the true sense, only tryptophan, lysine and methionine have reached the level of industrial production (Table 2).

## ORGANIC ACIDS BY FUNGI

Wide varieties of organic acids are produced by fungi through industrial fermentation and have much importance in industry (Table 3).

**Table 3.** Organic acids by fungi

Organic acids	Fungus	Uses
Citric acid	<i>Aspergillus niger</i> , yeast	Food industry, acidulant, nutrition enhancer, preservative, stabilizer, emulsifier, liquid wash
Gluconic acid	<i>Aspergillus niger</i>	Cleaning agent
Malic acid	<i>Schizophyllum commune</i> , <i>Pichia</i> sp.	Acidulant and preservative
Fumaric acid	<i>Rhizopus</i> sp.	Food ingredient
Lactic acid	<i>Rhizopus</i> sp.	Food acidulant
Kojic acid	<i>Candida brumptii</i>	Antibiotic, insecticidal properties

## FUNGAL LIPASES

Lipids are soluble in organic solvents and insoluble in water and are derived from living organisms including fungi. Fungi had become important in 19<sup>th</sup> century as some strains of yeasts and filamentous fungi were known to produce relatively high amounts of linolenic acid, ergosterol, extracellular cyclolipids and other such fatty acids.  $\gamma$ -linolenic acid is of pharmacological interest as it is a precursor to prostaglandins of the PEGA series. *Mucor javanicus* and *Mortierella* sp. are considered as potential producers. Extracellular lipases are glycoproteins and are produced by *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Geotrichum*, yeast and others. Lipases are a group of the sterols, one of the most commercially produced products of fungal origin. The lipase gene from *Rhizomucor micheli* was cloned and sequenced (Boel *et al.*, 1988).

## CAROTENOIDS FROM FUNGI

Carotenoids represent one of the most important and widespread groups of naturally occurring pigments. They are responsible for yellow and red colours. They have been widely implicated in photo protection and are metabolized to sex hormone, trisporic acid in the *Mucorales*. Synthetic carotenoids are used as colorants. The retinoids are used as colorants and are also used as anticancer agents. Carotenoids from around 500 plants are identified. They

**Table 4.** Fungal carotenoids

Fungus	Carotene
<i>Phycomyces</i> sp.	phytoene
<i>Cantharellus</i> sp.	$\beta$ -carotene
<i>Blakslea trispora</i>	$\beta$ -carotene
<i>Rhizophlyctis rosea</i>	$\gamma$ -carotene
<i>Phaffia</i> sp.	Astaxanthin
<i>Gibberella fujikorii</i>	Xanthin
<i>Epicoccum nigrum</i>	Rhodoxanthin

are not ubiquitous and around 200 fungal species are reported to yield carotenoids.  $\beta$ -carotene is the most common pigment in *Phycomyces*, *Allomyces*, *Neurospora*, hymenomycetous fungi and others (Table 4).

## NUCLEIC ACIDS AND NUCLEOTIDES

Nucleic acids and nucleotides of fungi are of commercial importance as they facilitate genetic modification of fungi to obtain useful properties for other purposes. Studies on molecular aspects using *Neurospora*, *Saccharomyces cerevisiae*, *Emericella nidulans*, *Rhizopus sexualis* and some basidiomycetous fungi revealed in depth information on sexuality of fungi, mendelian ratio, gene-gene for hypothesis, heterothallism, genetic control, mutations, genetic complimentary and other related aspects.

## ALKALOIDS

Ergot fungi grow on wild grasses and cereals which form sclerotia and these contain pharmacologically active ergot alkaloids. *Claviceps* spp. possesses lysergic acid derivatives, ergotamine, ergosine, ergerstine, ergokryptine, ergocornine and ergostine alkaloids. These are used as medicine in small quantities to treat blood pressure, migraine, vascular headaches, breast and prostate cancer and control of blood flow during child birth. Smaller quantities serve as medicines including hallucination and heavy doses are not advisable.

## ANTIBIOTICS FROM FUNGI

Penicillin, the oldest antibiotic was produced from *Penicillium chrysogenum* by Alexander Fleming. Penicillin proved not to be a single compound or even unique series of compounds. The cephalosporin antibiotics were discovered later and these proved to be structurally related to penicillin. These two antibiotics are unusual molecules due to the presence of a four membered  $\beta$ -lactam ring systems. The microbially synthesized antibiotics are called natural components, which can be directly applied clinically or can be further mediated

**Table 5.** Some antibiotics from fungi

Fungus	Antibiotic
<i>Cephalosporium acremonium</i>	Cephalosporin C
<i>Emericellopsis</i> sp.	Penicillin N
<i>Fusidium coccineum</i>	Fusidic acid
<i>Paecilomyces variotii</i>	Variotin
<i>Penicillium chrysogenum</i>	Penicillin G
<i>Penicillium patulum</i>	Griseofulvin
<i>Tolyocladium inflatum</i>	Cyclosporin



through chemical or enzymatic methods. Cyclosporine is used in organ transplantation. Some common antibiotics produced by fungi are listed in **Table 5**.

## MUSHROOMS

Mushrooms are the fleshy spore bearing structures of some fungi. The majority of the mushrooms belong to the class *Basidiomycota* and a few to *Ascomycota*. The sporophores are variously shaped. They may be stalked with the pileus and gills are situated on the underside of the pileus e.g., *Agaricus* sp. Numerous pores may be present in the sporophores e.g., *Boletus* sp. sporophores may be covered with teeth like structures e.g., *Hydnum* sp. sporophores which are angiocarpous (closed) and open by an apical pore at maturity are produced in *Gasteromycetes* e.g., *Lycoperdon* sp. Other fleshy fungi like *Morchella* sp. belong to *Ascomycota* producing ascospores in a sponge like sporophores. Around 200 species are edible throughout the world but all of them are not cultivated. Mostly basidiomycetous mushrooms are cultivated for centuries like white button mushroom (*Agaricus bisporus* or *A. bitorquis*), paddy straw mushroom (*Volvariella* spp.), oyster mushroom (*Pleurotus* spp.) and shiitake mushroom (*Lentinula edodes*).

Recently some other mushrooms are also grown in different parts of the world. They are black ear mushroom (*Auricularia polytricha*), nameko mushrooms (*Pholiota nameko*), enokitake mushroom (*Flammulina velutipes*), giant fungus (*Stropharia rugoso-annulata*), truffles (*Tuber melanosporum*), jelly fungus (*Tremella fusiformis*) and milky white mushrooms (*Calocybe indica*).

Sufficient food supply is the need of the growing population. It is essential that search must be made for alternate sources of energy and food. Mushrooms contain all essential amino acids, elements, proteins, vitamins and others. They contain more water and lesser values of fat, starch and cholesterol. Mushrooms being low in calories form healthy diet for people suffering from diabetes and cardiac problems. To solve the problem of protein deficiency, hunger and malnutrition, cultivated mushrooms become a great source besides being a good source of energy. The following table gives an insight into different nutrients present in edible mushrooms (**Table 6**).

**Nutritional value of mushrooms:** Mushrooms contain 2 to 4% of proteins on an average on fresh weight basis. These proteins consist of all the amino acids required by human body. Being a protein food, consumption of mushroom prevents protein malnutrition and reduces the rate of diseases like kwashiorkar, marasmus leading to anaemia, etc. in human beings. Tryptophan and lysine, which are absent in cereals, the staple food of Indian diet are present in high concentrations in mushroom protein.

**Table 6.** Composition of common edible mushrooms (per 100g of fresh weight)

Mushroom	Moisture	Protein	Fat	Carbohydrate	Fiber	Ash	Calories
<i>Agaricus bisporus</i>	90.1	2.9	0.3	5.0	0.9	0.8	36
<i>Volvariella volvacea</i>	90.1	2.1	1.0	4.7	1.1	1.0	36
<i>Pleurotus sajor-caju</i>	90.2	2.5	0.2	5.2	1.3	0.6	35

Methionine and cysteine present in low amounts in mushrooms can be compensated by cereals. The quality of mushroom protein is intermediate between vegetable and animal protein. Also many crops of mushrooms can be produced in a year, thus compensating for the protein production.

Only 0.4% of fat on fresh weight basis is present in mushrooms. This fat consists of free fatty acids, monoglycerides, diglycerides, triglycerides, sterols, sterol esters and phospholipids. About 72% of the fatty acids are unsaturated fatty acids, especially linoleic acid. Cholesterol, responsible for heart attack is absent. Instead, ergosterol that can be converted to Vitamin D by the human body is present. Mushrooms consist of 2-5% carbohydrates on fresh weight basis. Fresh mushrooms consist of 0.95% mannitol, 0.28% reducing sugars, 0.59% glycogen and 0.91% hemicelluloses. Starch is completely absent hence suitable for diabetic patients. Mushrooms are rich in fibre. The fibre content in *Pleurotus* spp. ranges from 7.4 to 27.6%, in *Volvariella* sp. 4 to 20% and in *Agaricus bisporus* 10.4% on dry weight basis. Mushrooms have vitamin C (ascorbic acid) and B-complex group of vitamins like biotin, niacin, pantothenic acid, riboflavin, thiamine, folic acid and B-12. The two vitamins, folic acid and B-12 lacking in many vegetables and essential for pregnant and lactating women are present in mushrooms. Ascorbic acid is present in *Agaricus bisporus* (81.9 mg/100g dry weight) and *Volvariella volvacea* (20.2mg/100g dry weight). Vitamin A, D, E and K are absent in mushrooms but ergosterol present in mushrooms can be converted to Vitamin E by the human body. Mushrooms have good amount of minerals. Potassium is present in high amounts followed by phosphorus, sodium, calcium, and magnesium. Minerals like K, P, Na, Ca and Mg comprises about 56 to 70% of the total ash content. Potassium alone accounts for about 45% of total ash content. Na, Ca are in equal amounts in all other edible mushrooms except in *Lentinula edodes* where calcium is in large amounts. Due to high potassium and low sodium ratio mushrooms become the choice of patients suffering from hypertension. Other minerals like copper, zinc, iron, manganese, molybdenum and cadmium are present in minor amounts. Usually microorganisms consist of high content of nucleic acid. One can take 4g of nucleic acid per day of which only half should come from microbial source. Of the four edible mushrooms studied *Pleurotus sajor-caju* contains the highest amount of nucleic acid and one can consume 392.5g of fresh mushrooms of *P. sajor-caju* per day. Other mushrooms which are low in nucleic acid content can be consumed in higher quantities.

**Medicinal importance of Mushrooms:** Mushrooms have been used in medicine since ages. Medicinal value of mushrooms was mentioned even in Vedas. The mushrooms consist of an enzyme, polyphenol oxidase responsible for proper metabolism and good health. The mushrooms are antiviral, antitumourous and antibacterial. Haematological effects are between the fungal protein, the lectin and the surface glycoproteins of red blood cells. Lectins have been isolated from few edible mushrooms. Such lectins were purified from *Agaricus campestris* and *Flammulina velutipes*. *Volvariella volvacea* produces volvatxin-A, which reduces haemolytic effect towards 'O'

group red blood cells. *Fomes ignarius* and *F. fomentarius* are used for blood coagulation. *Polyporus officinalis* when applied externally stops bleeding. Antiviral properties of mushrooms were reported from *Lentinula edodes*, *Boletus edulis* and *Calvatia gigantea*. Lentinan sulphate is inhibitory to AIDS. *Ganoderma lucidum* is marketed in the form of pills. This mushroom consists of immune regulating compounds and thus helps in controlling AIDS. Some *Ganoderma* spp. are used as anticancerous agents besides being used in cosmetics and as ornaments. Hence, it is called "Longevity Mushroom" in Korea. Water extract of *Grifola frondosa* has been reported to kill HIV virus in human beings. This was found to be equally effective to that of widely used AZT drug for AIDS. Mushrooms like *Pleurotus*, *Agaricus bisporus*, *A. blazei* and *Grifola frondosa* are reported to be antitumourous. The antitumour compounds like 'Lentinan' from *Lentinula edodes*, Calvacin from *Calvatia gigantea*, Flammulin and Flammutoxin from *Flammulina velutipes* and volvatoxin from *Volvariella volvacea* have been reported. They inhibit the respiration of tumour cells. Lentinan (antitumourous) and rentinan (anticancerous) are being produced by Ajinomoto Co., Japan.

Cardiotoxic proteins isolated from *Flammulina velutipes* and *Volvariella volvacea*, *Auricularia polytricha*, *Agaricus bisporus* and *Lentinula edodes* have been reported to show hypolipidemic effect, a property of reducing cholesterol level in the blood. Liquid extracts of *Pleurotus sajor-caju* is reported to reduce the Glomerular Filtration Rate (GFR) in rats. GFR reduction reduces the rate of nephron deterioration and increases the life span of chronic renal failure patients. Edible mushrooms having toxic effects are very rare. *Agaricus bisporus* consists of agaritine that shows negative effects in large doses in mice but its health hazard in man is negligible. Hallucination resembles alcohol intoxication. Some hallucinogenic mushrooms are *Amanita muscaria*, *Stropharia* sp. and *Psilocybe* sp. They contain psilocybin, a substance responsible for hallucination and is used in the treatment of mental disorders.

Inhalation of spores of *Pleurotus* sp. develop allergy in some people. The symptoms are fatigue, fever, sinus pressure, joint pains, cough and nausea. The basidiospores deposited in smaller bronchi induce asthmatic symptoms which last for 1 to 2 days or up to one week and disappear without treatment. Using respiratory masks inside the growing rooms gives relief to the persons suffering from allergy. *Polyporus officinalis* is used to cure chronic diseases of lungs and breast, gout, jaundice, rheumatism, intestinal worms and night sweating in tuberculosis, etc. *Lycoperdon giganteum* is used for surgical dressing. *Calvatia gigantea* is used for anesthesia. *Auricularia auricula* is used as a gargle for throat inflammation and also for inflamed eyes. *Amanita muscaria* is used for epilepsy, swollen glands, and rheumatoid arthritis and heart ailments. The two compounds muscinol and ibotenic acid extracted from *A. muscaria* cure the mental disorder schizophrenia. *Flammulina mellae*, *F. odilpis* and *Agaricus bisporus* are antibacterial and are active against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. *Lentinula edodes* and *Oudemansiella canarii* are antifungal in nature. Many mushrooms are used in

Ayurveda also.

## GROWTH PROMOTERS

A total of 71 Gibberellins are known to be produced by fungi and are mainly from *Gibberella fujikorii* and *Fusarium moniliforme* using submerged fermentation process. Around 27 different cytokinins are chemically identified. *Uromyces*, *Trichoderma* sp. and others also yield cytokinins.

Auxins include a variety of compounds that are generally acids with an unsaturated cyclic nucleus or their derivatives. Auxins are also produced by bacteria, fungi, actinomycetes, yeast, algae and mycorrhizal fungi. Fungi like *Aspergillus*, *Fusarium*, *Curvularia*, *Exobasidium*,

**Table 7.** Growth promoters by fungi

Growth promoter	Fungus
Gibberellic acid	<i>Gibberella fujikorii</i> , <i>Fusarium moniliforme</i>
Cytokinins	<i>Agaricus bisporus</i> , <i>Exodium mirtilli</i> , <i>Taphrina deformans</i> , <i>Puccinia carthami</i>
Auxins	<i>Aspergillus</i> sp., <i>Fusarium</i> sp., <i>Boletus</i> sp.

*Rhizopus*, *Gibberella*, etc. are known to produce Indole acetic acid. Among these Gibberellic acid is the only one produced commercially through fermentation. Some of the growth promoters produced by fungi are listed in the table below (Table 7).

## FUNGIAL MYCOHERBICIDES

Mycoherbicide is a product of fungal origin which is usually pathogen based formulation that prevents, controls or kills the targeted weed interfering with crop growth and yield. It is a biocontrol agent that may be weed specific. Around sixteen mycoherbicides have been registered. Collego produced by *Colletotrichum gloeosporioides* is used against sicklepod weed, Devine from *Phytophthora palmivora* is used for the control of milk weed.

## ETHANOL PRODUCTION BY YEAST

Ethanol is the resultant product of common chemical transformations. Optimal conversion of carbohydrates to ethanol requires cells that are tolerant to high concentration of both substrate and product and are able to efficiently produce ethanol at relatively high temperatures *Saccharomyces* sp. and in particular *S. cerevisiae* are the most ethanol tolerant Eukaryotes. Ethanol is the product of glycolysis and is known to induce sugar fermentation and cause other effect in yeast cells. However, huge literature has accumulated on ethanol production by yeast and this commercial product has gained importance in world trade. It is commonly understood that 50% fungal biotechnology is because of yeast. Growth and fermentation partly limited by yeast strains used in commercial alcohol fermentation are usually polyploid or aneuploid, non-mating and unable to separate. Ethanol tolerance is influenced by environmental and nutritional conditions

and cell membrane seems to be the determinant for alcohol production. Further genetic improvement of industrial yeast requires the transfer via conjugation or protoplast fusion. These aspects need to be taken care for sustained commercialization of ethanol production by yeast species.

### BIODEGRADATION OF XENOBIOTICS

With industrialization and the extensive use of pesticides in agriculture, the pollution of the environment with manmade (synthetic) organic compounds has become a major problem. Many of these novel compounds introduced into nature are called xenobiotics and a large number of them are not easily degraded by the indigenous micro flora and fauna. The list of xenobiotics is very long and some of them are directly applied to nature in the form of pesticides or fertilizers, some others are released as industrial waste products. The toxic pesticides are of two types: biodegradable and non-biodegradable (recalcitrant). A biodegradable pesticide can be converted by microbial action into a nontoxic compound within a few months where as a recalcitrant chemical may remain in nature for several years in the toxic form. Indiscriminate use of chemical pesticides particularly has affected man and his environment. Some of the pesticides such as Parathion, Aldrin, Chlordane, DDT, Diuron, and Endrin are known to persist in the environment for 14- 16 years. A persistent pesticide has a special position in environmental pollution because it may remain in soil long enough a) to be assimilated by plants and accumulate in edible portion, b) to adhere to edible portions of root crops, c) to be transported with eroding soil particles to nearby waterways, or d) to accumulate in earthworms and then show up in high levels in birds feeding on the worms (Alexander, 1961).

It is well known that microorganisms play an important role in the degradation and detoxification of many pesticides in the environment. The pesticide degrading characters of many microbes have been located to plasmids. Many fungi use pesticides as substrate, either metabolizing the molecules or using them as nutrients. Species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Glomerella*, *Mucor*, *Rhizopus*, *Penicillium*, *Rhizoctonia* and *Trichoderma* are among the fungi which modify one or more of the synthetic chemicals. The fungi are used as mycelium or cells to treat chemical pollutants. Alternatively fungal enzymes either in a free form or immobilized on a suitable substrate could be used to treat pollutants.

*Phanerochaete chrysosporium* has been shown to degrade a number of toxic xenobiotics such as aromatic hydrocarbons (Benzo alpha pyrene, Phenanthrene, Pyrene), chlorinated organics (alkyl halide insecticides, Chloroanilines, DDT), Pentachlorophenols, Trichlorophenol, Polychlorinated biphenyls, Trichlorophenoxyacetic acid), nitrogen aromatics (2,4-Dinitrotoluene, 2, 4, 6-Trinitrotoluene-TNT) and several miscellaneous compounds such as laccases, polyphenol oxidases, and lignin peroxidase play a role in the degradative process. In addition a variety of intracellular enzymes such as reductases, methyl transferases and cytochrome oxygenase are known to play a role in xenobiotics degradation. Biodegradation takes two forms;

the most desirous from an environmental point of view is the mineralization of the substrate to its constituent elements. This is most likely to occur when the molecule can be utilized as a source of carbon and energy by competent microorganism.

Several factors are known to influence the fate of pesticides in soil viz. chemical decomposition, photochemical decomposition, microbial decomposition, volatilization, movement in soil, plant or organism uptake and adsorption. The fungi along with bacteria and actinomycetes are capable of breaking down the organic herbicide molecule resulting in deactivation of the compound.

Pentachloronitrobenzene (PCNB) is a common fungicide used in the control of soil-borne pathogens of plants. This fungicide is gradually converted to pentachloroaniline (PCA) in most soils and the conversion is greatly enhanced by submergence of soil in water. In sterilized soil, PCNB remains unchanged while in unsterilized soils, loss of PCNB is accompanied by the increase in PCA indicating the role of microorganisms in the degradation of the fungicide.

The herbicide atrazine undergoes degradation in soil by two routes. One route involves replacement of the 2-chloro substituent by a hydroxyl group. A second route is mediated by a soil fungus (*Aspergillus fumigatus*) which involves dealkylation to give rise to 2-chloro-4-amino-6-isopropylamino-s-triazine and 2-chloro-4-ethylamino-s-triazine. The herbicides resistant to degradation by microorganisms, their accumulation and persistence pose serious residue hazard. Application of farmyard manure enhances certain microbial activity which in turn degrades the herbicide at a faster rate.

### DECOMPOSITION OF LITTER

Decomposition is the natural process by which large organic materials and molecules are broken down into simpler ones. Microorganisms including fungi play a fundamental role in the global recycling of matter. They degrade an immense variety of complex organic compounds and release carbon, nitrogen, phosphorus and sulfur to be reused by living organisms. In the absence of microbial degradation of organic matter especially plant litter often referred to as 'nature's garbage' would accumulate in vast amounts. Litter is defined as dead plant material not attached to a living plant. Decomposition may signify the mechanical disintegration of dead plant material and the breaking down of complex organic molecules to carbon dioxide, water and other nutrients. Decomposition of organic matter due to microbial activity is beneficial to man's interests. It benefits man in three very important ways.

1. Organic debris is continuously being removed from man's environment.
2. Large amount of carbon dioxide is released into the atmosphere and made available again for use by green plants.
3. Humus, a very important soil constituent is formed from waste organic material through the activities of microorganisms.



These three processes are of immense value to man. Were it not for the ability of microorganisms and fungi to decompose waste organic material, the earth in a very short time would be covered to an astonishing depth with an accumulation of vegetable debris and other waste products that had their origin either directly or indirectly in plant material. If fungi and bacteria were not active in the destruction of leaves at the end of the growing season the fallen dead leaves would accumulate to such an extent that the tree might be completely covered. An incalculable amount of organic material is decomposed annually through the activities of microbes and fungi. Man is greatly benefited by the unceasing decay activities of fungi and bacteria.

Plant litter is composed of six main categories of chemical constituents: (i) cellulose, (ii) hemicelluloses, (iii) lignin, (iv) water soluble sugars, (v) ether and alcohol soluble constituents including fats, oils, waxes, resins and many pigments and (vi) proteins. The breakdown of these constituents is affected as a sequence of specific reactions with the enzyme systems of specific organisms. Because many species have broadly similar enzyme complements they can be classified in physiological groups which follows succession as litter decomposers.

Three separate simultaneous processes can be distinguished during litter decomposition. i). The constituents of plant litter disappear under the influence of microbial enzymes. ii). At the same time new microbial cells are synthesized. iii). Certain end products of the breakdown are excreted into the surroundings.

If the substrate is too complex to penetrate, the compound must be first transformed into simple molecules to allow the organisms to derive energy from the oxidations. Insoluble polysaccharides are commonly hydrolyzed to soluble, simple compounds. Mineralization is a convenient term used to designate the conversion of organic complexes of an element to the inorganic state. Many enzymes such as amylase, cellulase, pectinase, lignase, laccase, xylanase, protease, lipase and others play vital role in the breakdown of organic debris/litter.

#### **TRICHODERMA AS BIOCONTROL AGENT**

*Trichoderma* spp. are free living fungi that are present in all soils, and are the most prevalent culturable fungi. Many species are avirulent plant symbionts. This results in forming mutualistic endophytic relationships with several plant species. There are more than 100 species in the *Trichoderma* genus. The avirulent plant symbionts can be established as long lasting colonizers around the root surface. *Trichoderma*-plant root association enhances root growth, development and crop productivity by increasing resistance to abiotic stress, seedling establishment, nutrient uptake, enhancing translocation and nutrient use efficiency. They produce or release a variety of compounds that induce localized or systemic resistance responses, thus making them non pathogenic to plants. These root associated fungal associations cause substantial changes to the plant proteome/genome and also numerous classes of plant pathogens whose responses are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance. Several strains of *Trichoderma* have been

developed as biocontrol agents against fungal and bacterial diseases of plants. The various mechanisms include antibiosis, parasitism, inducing host-plant resistance, and competition. Most biocontrol agents are the species *T. harzianum*, *T. hamatum*, *T. asperellum* and *T. viridi*. *Trichoderma* and its beneficial effects popularized its use as biofungicide and biofertilizer in annual and perennial crops. (Nagamani and Sarojini, 2012)

#### **SINGLE CELL PROTEIN**

Some fungi such as yeast and dried biomass of certain fungi are consumed as a protein source in human food and animal feed. Such proteins are called as mycoprotein. These fungi are grown on cheap raw materials and mycoproteins have fewer amounts of nucleic acids than bacteria. The mycoprotein are easily digestible and accepted by humans, hence dried biomass is consumed (Baker's yeast). *Saccharomyces fragilis*, *Candida utilis*, *Candida lipolytica*, *Rhodotorula glutinis*, *Torulopsis spp.* and *Fusarium graminearum* and others serve as source for single cell protein and million tones of yeast protein is manufactured all over the world per year. In India village folk consume liquid collected from toddy in the early morning hours before sunrise as nutritive drink as it contains yeast (*Saccharomyces cerevisiae*). Thaumatin is a product from genetically modified yeast called sweet protein and is used as a substitute for sugar based sweeteners for diabetics. Edible mushrooms such as button mushroom form rich source of single cell protein. However, there are difficulties in converting lignocelluloses into fungal biomass indicating the essentiality of finding out an organism which converts lignocelluloses effectively into fungal biomass rich in essential amino acids. This requires genetic improvement of fungal strains. Thus fungi serve the purpose of removing protein deficiency besides being a source of natural protein.

#### **TRANSFORMATION OF STEROIDS**

Steroids are a group of cyclical organic compounds with characteristic arrangement of seventeen carbon atoms in a four-ring structure linked together from three 6-carbon rings followed by a 5-carbon ring and an eight-carbon side chain on carbon 17. Steroids are used as signaling molecules. Fungi are used for steroid biotransformation to prepare specific derivatives, the production of which is difficult by traditional synthetic methods.

Steroid compounds are the widely marketed products by the pharmaceuticals. The important application of fungal technology is the steroidal drug production and intermediaries via industrial processes. In steroid biotransformation, there is a necessity of biocatalysts, which offer high efficiency region, stereo selectivity and economic routes. Many fungi such as *Fusarium fujikori*, *Rhizomucor pusillus*, *Aspergillus niger*, *Penicillium spp.*, *Aspergillus ochraceus* and others are employed. The hydroxylation of progesterone by fungi has been reported by many fungi.

Caffeine is the major alkaloid in coffee and theanine in tea besides the presence of other methyl xanthenes in smaller quantity. The degradation of caffeine and such alkaloids by fungi and microbes has great potential in developing

decaffeination process replacing the use of toxic organic solvents. Further it also helps in the decontamination of these compounds in the environment and production of high value alkyl xanthenes.

### OTHER BIOTECHNOLOGICAL APPLICATIONS

**Coal solubilization:** Coal is an important mineral having many industrial uses and contributes to the economy of any country in the world. Biological solubilization of coal is important because it occurs at virtually ambient temperatures and pressures and changes solid coal properties to a liquid coal with little loss in total energy content. Solubilization of coal has been demonstrated by *Trametes versicolor*, *Trichoderma atroviride*, *Trametes* sp., *Poria monticola* and others. Filamentous fungi, yeasts and bacteria were isolated by some researchers that grow on coal as their carbon source. It was documented that some basidiomycetes were able to convert leonardite, a low rank coal to a black fluid. It appears that lignin peroxidase, manganese peroxidase and laccase appear to be involved in the depolymerization of raw coal. Brown coal gets degraded by some basidiomycetes including *Phanerochaete chrysosporium*.

**Mycoinsecticides:** Some fungal groups such as Entomophthorales and few others grow and multiply on insects. Some of the fungi are used as an insecticide, hence called mycoinsecticide. In view of growing population, food security needs priority, hence crop protection assumes importance. Around 750 fungi that are pathogenic to insects are reported and of which 12 have been recognized as potential insecticides.

*Beauveria bassiana*, and *Metarhizium anisopliae* are the two major and prominent species and these are applied as spray or in a commercial form. The fungal hyphae pierce the outer layer of insect, spread and eventually kill the insect. *Metarhizium anisopliae* acts as a low power insecticide. However, the insecticide strength can be increased by a scorpion species. *Beauveria bassiana* causes white muscardine disease. The fungus invades, proliferate and reproduce leading to cell death due to nutrient depletion of the haemolymph or toxemia by fungal toxic metabolites. Mycoinsecticides are the easily available cheap chemical pesticides of the market. The commercial success is dependent on the efficacy of the fungal strain. Advanced research is going on in the commercial success with genetic and physiological engineering.

**Fungal dyes:** Fungal pigments are the colour molecules found in fungi and lichens. Diversified mushroom colours are the greatest source for the extraction in water and can be used to dye protein fibres like wool and silk besides some being used as paint. Yellow pigment from *Gymnopilus spectabilis*, red pigment from *Cortinarius neosanguineus* and *Monascus* sp., a powerful purple dye from *Hapalopilus nidulans* and also red pigment of fly agaric are used in textile industry.

**Fungal vitamins:** *Saccharomyces cerevisiae* is the source for brewer's yeast and is used to make beer. Brewer's yeast is a rich source of minerals including chromium that helps in to maintain normal blood sugar level. It also contains selenium, protein and  $\beta$ -complex vitamins. *Monascus*

*purpureus* is the red yeast that is used to ferment rice giving red colour, hence called red yeast rice. It is used as a food preservative, food colorant and ingredient of rice in China and other countries. In China, Japan and Asian communities in USA consume 14-55gm of red yeast rice per person. The Chinese have used it for improving blood circulation. Yeast/Fungal detox is available as commercial product that promotes optimal balance of microflora, boosts the immune system, stimulates detoxification of liver, supports digestive system. Yeast is the great source of vitamin B12 and other B vitamins. Nutritional yeast has strong flavor. Riboflavin (vitamin B2) an essential vitamin is commercially produced from *Eremothecium gossypii* because it cannot be synthesized by vertebrates.

**Fungal chitin:** Chitin is the cell wall component of many fungi and forms protective structure. Chitin has filled the need to build a resistant cell wall. Isolation and study of chitosomes have provided valuable information on the mechanism of chitin fibrillogenesis. Presence of chitin is the unique property of fungi and insects, which has elevated the fungi to the level of a kingdom Mycota along with some other characters. The genes responsible for the synthesis of chitinase offer resistance to many plant pathogenic fungi and other microbes.

**Mycoremediation:** The decontamination of soil and water from pollutants using microbes, fungi and plants is known as bioremediation. When the contaminated material is treated on site it is *in-situ* bioremediation and when material is physically removed to be treated elsewhere it is *ex-situ* bioremediation. Microorganisms individually cannot degrade most hazardous compounds, therefore a consortium of microorganisms is used for complete bioremediation (Singh *et al.*, 2014). *Pseudomonas aeruginosa* and mycorrhizal rye grass is used for pesticide degradation, cow dung for benzene, *Pseudomonas putida* for benzene, toluene and o-xylene biodegradation. White rot fungi digest lignin by means of enzymes and give a bleached appearance to wood from undissolved cellulose, hence their name. Whereas, brown-rot fungi degrade cellulose, leaving lignin as brownish deposit. *Phanerochaete chrysosporium* is the commonly used fungus for bioremediation. Species of *Trametes*, *Pleurotus*, *Aspergillus*, *Penicillium* and others are also employed. It has been demonstrated by Singh (2006) that soils contaminated with oil get decontaminated by using oyster mushroom.

**Fungal genome mining:** The metabolism of a living organism possess primary metabolism which provides the living cells with chemical precursors that are essential for growth and reproduction. However, the secondary metabolism does not have any function for cell growth but it remains as a constant source of drug leads with more than 40% of new chemical entities. More than 23,000 bioactive microbial products are reported. Though fungi produce metabolites, the biosynthetic potential of these organisms is greatly underestimated by an order of magnitude.

Analyzing the increasing number of sequenced genes indicate that fungi encode the genetic information for the biosynthesis of many more yet unknown compounds. Possibly this can be achieved bioinformatically through



sequencing genome for the preservation of characteristic biosynthetic genes. The genomes of *Emericella nidulans*, *Aspergillus fumigatus* and *A. oryzae* have been sequenced. The gene products of *Emericella nidulans* suggest that it has potential to generate up to 32 polyketides, 14 non-ribosomal peptides and two indole alkaloids. Similarity *Saccharomyces cerevisiae* has been worked out thoroughly for genomics and gene mining besides conserving pumilio family genes from yeast to man. Many more surprises are expected to be reported in near future (Matsushima *et al.*, 2007).

**Mangrove and Marine fungi:** Mangroves are intertidal forest wetland confined to the tropical as submerged regions. It is a dynamic ecotone between land and marine habitats. Around 1500 marine fungi are reported mostly belonging to *Ascomycota*, *Basidiomycota*, Mitosporic fungi and Zoosporic fungi. Mangrove fungi constitute the second largest ecological group of the marine fungi. Saprophytic fungi are fundamental to decomposition and energy flow of mangroves. There are many bioactive compounds synthesized by marine fungi which need elaborate studies (Raghukumar, 1996). A total of 721 obligate marine fungi are reported (Raghukumar, 2006). It is surprising that till date less than 5% of marine fungi have been investigated for their secondary metabolite chemistry. Most of the described fungi were isolated from driftwood, mangrove wood, sea mud, sand and coastal marsh grass. Marine-derived fungi have been shown to be a tremendous source for new and biologically active secondary metabolites which is reflected by the increasing number of published literature dealing with compounds from this group of fungi.

**Mycorrhiza in agriculture and forestry:** Soil is a dynamic medium for millions of microorganisms. Saprophytic, biotrophic and symbiotic fungi are found associated with wide variety of soils. The rhizosphere is a specialized ecological niche harboring countless microorganisms including symbiotic and beneficial microbes. The soil stratum contains undecayed litter or partially decomposed litter on the top followed by humus, sand and silt, clay and mineral deposits, slightly broken bedrock and unweathered parent rock at the bottom. The top 6-10 cm layer of soil contains mineral and organic matter, hence considered as fertile zone of high microbial activity. Several physical parameters such as moisture,  $p^H$ , temperature, nitrogen, phosphorous, organic matter and soil type/soil texture influence the occurrence, distribution, seasonal variation, quantitative and qualitative account of fungi and other microbes.

India with its varied vegetational types, climatic conditions, geographical regions, agroclimatic zones and soil types harbor a wide variety of microorganisms. Mankind is dependent on both harmful and beneficial microbes. Biodiversity of microorganisms including fungi aroused worldwide interest because of international agreements on IPR and patenting. Microbial diversity forms an important resource material for industry, agriculture, medicine, pharmaceutical, environment management, forestry, etc. The fungal population is estimated to be 1.5 million, of which more than 93,843 fungal species are known and much remain unidentified

(Kirk *et al.*, 2008). From India 29,000 fungal species are reported, thus contributing to one-third of biodiversity status (Manoharachary *et al.*, 2005) among identified fungi of the world.

Besides the disease causing microbes several symbiotic groups, phosphorous solubilizers, plant growth promoters and other such beneficial important microorganisms are reported from different soils. Balanced microbial systems contribute to the sustainability in agriculture, forestry and range management. In this regard mycorrhiza has a substantial role. The term mycorrhiza (fungus root) was coined by Frank (1885). Harley and Smith (1983) have defined mycorrhiza as an association between fungal hyphae and roots of higher plants concerned with absorption of mineral substances from the soil. Brundrett (2004) has defined as a symbiotic association for one or both partners between a fungus and root of living plant that is primarily responsible for nutrient transfer. Volume of soil that the plant roots explore is greatly enhanced by mycorrhizal fungal hyphae. A considerable benefit to both the plant and fungi is provided by majority of mycorrhizal associations. Mycorrhiza is considered as a key factor in the below ground network essential for ecosystem function. Mycorrhizal associations are known to benefit plants under conditions of nutritional and water stress and pathogen challenge.

Mycorrhizas were earlier classified into arbuscular, ectomycorrhizal and orchid mycorrhizal association based on the relative location of fungi in the roots. The basic types of mycorrhizas now established are: 1. Ectomycorrhiza, 2. Arbuscular mycorrhiza, 3. Orchid mycorrhiza, 4. Ericoid mycorrhiza, 5. Monotropoid mycorrhiza, 6. Arbutoid mycorrhiza, 7. Miscellaneous types of mycorrhiza

Besides the morphological, anatomical, histochemical, biochemical tools, molecular and genetical tools are also used in identifying them and to explore the structural and regulatory genes in both fungus and plant and permit mycorrhiza formation. Of the seven types of mycorrhizas, the two prevalent mycorrhizal types are the ectomycorrhizas common with woody species related to forestry and the arbuscular mycorrhizas more often associated with the herbaceous plants with relevance to horticultural, ornamental, medicinal and crop plants. A large proportion of land area in India shows clear evidence of soil degradation which in turn is affecting the countries productive resource base which is because of salinity, alkalinity, soil erosion, water logging, etc. The fundamental problem that the country is facing today is the rapidly increasing pressure of population on the limited resources of land. In order to meet the pressure of population it is essential to efficiently manage the agriculture inputs for sustaining high crop productivity on long term basis with minimum damage to environment. In order to reduce cost of agrochemicals and harm rendered by them biofertilizers are used. Mycorrhizas help the plants to acquire mineral nutrients from the soil, especially immobile elements such as P, Zn, Cu but also mobile ions such as S, Ca, K, Fe, Mg, Mn, Cl, Br and N. They also help in increasing the extent of soil particle aggregation.

Mycorrhizal association is known to offer resistance in

host plants to drought and plant pathogens. Mycorrhizal fungi improve host nutrition by increasing the 'P' delivery and other minerals to roots and plants. Further, most of the economically important plants have been found to be mycorrhizal, and the subject is currently gaining much attention in agriculture, horticulture and forestry. Due to the unique ability of mycorrhiza to increase the uptake of 'P' by plants, mycorrhizal fungi have the potential for utilization as a substitute for phosphatic fertilizers. It is also known that Glomalean fungi existed four hundred million years ago and helped in the colonization of the land by primitive plants. Thus the primary land plant establishment was also due to mycorrhiza. The mycorrhizal symbiotic association appears to have evolved as survival mechanism for fungi and higher plants thus making each to survive in the existing environment of low temperature, soil fertility, drought, diseases, extreme environments and other stress situations. Mycorrhizas offer first line biological defense to host plants against stress for crops and forest trees.

Benefits derived from mycorrhiza by host plants:

1. Increased nutrient and water absorption through improved absorptive area.
2. Increased nutrient uptake, translocation of elements to host tissue and their accumulation.
3. Ectomycorrhizal fungi permeate the F and H horizon of forest floor and thus minerals get mobilized in these zones followed by their absorption before they reach sub soil system.
4. AM fungi are known to degrade complex minerals and organic substances in soil and thus make essential elements available to host plants.
5. Mycorrhizal association affords protection to host plant not only by creating a physical barrier but also by the secretion of antibiotics.
6. Mycorrhizal association helps in the production of growth hormones like auxins, gibberellins and growth regulators such as vitamin B.
7. Mycorrhizal fungi contribute to organic matter turnover and nutrient cycling in forest and crop land ecosystems and its biomass can account for 15% of the net primary production.
8. Increase the tolerance of the plant to adverse conditions.
9. Mycorrhiza help in soil aggregation, soil stabilization and add strength to soil fertility.
10. Mycorrhizas are not parasitic and are symbiotic. Therefore live hand in hand with other living organisms and are non-pollutants.

### FUNGAL ECOLOGY

Ecology is the study of organisms in relation to their environment. The population of organisms may interact in different ways e.g. commensalism, protocooperation, mutualism, neutralism, immensalism, parasitism and predation, etc. In fungi mostly autecological studies have been done. Fungi are ubiquitous and occur wherever decaying organic matter is present. Fungi could be

terrestrial or aquatic. Terrestrial fungi could be terricolous, humicolous, superstral, phoenicoid, carphophilous and fumicolous. Those growing on animals and human beings include dermatophytes and keratinophiles. Fungi inhabiting plants and their parts offer more diversity e.g. fructicolous, graminicolous, culmicolous, semicolous, fruticolous, ovaricolous, lignicolous, hydroxylophilic, fungicolous and agaricolous besides phylloplane, rhizoplane and mycorrhizal fungi. Population of fungi in the soil is extremely diverse and is influenced by a number of biotic and abiotic factors. Fungi are an integral part of one living world, so their activities have a direct bearing on human welfare, e.g. cause of allergies in humans, incidence of epiphytotics. Ecological studies of soil fungi have helped in understanding etiology of several crop diseases and their importance in decomposition of organic matter thus in cycling of nutrients.

### Hyphomycetous fungi for plant health and human wealth:

The fungal world includes molds, yeasts, mushrooms, puffballs, jelly fungi, bracket fungi besides many others. These fungi colonize diversified habitats in nature. A major proportion of fungi belong to Fungi Imperfecti or anamorphic fungi. Our knowledge of fungal diversity is based on observation, taxonomy, nomenclature, classification and identification. One of the important characters that distinguish the fungi from plants is the composition of their cell walls that are mostly made of chitin. Hyphomycetous fungi are nothing but asexual phases of either *Basidiomycota* or *Ascomycota*. The perfect stages of anamorphic fungi are hidden in the natural habitats and such stages need to be observed. Further hyphomycetous fungi reproduce asexually by conidia. The reorganization of the complex phenomena, conidiogenesis is certainly a great step towards a more natural classification. The hyphomycetous fungi play a significant role in the biodegradation and recycling of organic matter. They help in treatment of certain raw material and waste, and in breaking down of pesticides. Certain fungi are important in nutrition, industrial production, medicine, bio-control and also in disease protection. Thus hyphomycetous fungi have occupied a prominent place among fungi because of their biotechnological significance in industry, agriculture and medicine. From the point of numbers, the hyphomycetous fungi are represented by 1,800 genera and 16,000 species. Approximately 6,000 fungal species representing this group are reported from India. Around 5% of those fungi are found in culture. (Manoharachary, 2008).

**Thermophilic Fungi and prospects:** Temperature is one of the most important ecological factors that affect microbial activities and their distribution in nature. Microbial species exist in a great variety of environments with extremes of temperature, pH, moisture, pressure and several others. The occurrence is due to their genetic and/or physiological adaptations. Of the three domains of life, most of the thermophilic species described so far belong to Archaea and Bacteria. The maximum growth temperature limit for Eukaryota has been recorded as 62 °C. There are fewer than 50 species of thermophilic fungi which thrive at relatively elevated temperatures. These are common in soils and in habitats wherever organic matter

heats up due to decomposition. Thermophilic fungi have been isolated from manure, compost, industrial coal mine soils, beach sands, nuclear reactor effluents, Dead Sea valley soils, and desert soils of Saudi Arabia. Generally there is an inverse relationship between biological diversity and the adaptation required to survive in a specific habitat.

Thermophilic fungi are a small assemblage in Eukaryota, which have evolved strategies for growing at elevated temperatures up to 60–62 °C. During the last 50 years, many species of thermophilic fungi sporulating at 45 °C have been reported. The species capable of optimal growth at or beyond 40 °C are called thermophilic fungi/moulds. Much is known about the occurrence of thermophilic fungi from various types of soils and in habitats where decomposition of plant material takes place. These include: composts, piles of hay, stored grains, wood chip piles, nesting materials of birds and animals, snuff, municipal refuse, and others where the warm, humid, and aerobic environment provides the basic physiological conditions for their development. In these habitats, thermophiles may occur either as resting propagules or as active mycelia depending on the availability of nutrients and favorable environmental conditions. Soils in tropical countries do not appear to have a higher population of thermophilic fungi than soils in temperate countries as believed earlier. Their widespread occurrence could well be due to the dissemination of propagules from self-heating masses of organic materials. Thermophilic fungi are much more common in acidic thermal habitats than those of neutral to alkaline pH. These fungi constitute a heterogeneous physiological group of various genera in the *Zygomycota*, *Ascomycota*, *Basidiomycota* and Mycelia Sterilia. The occurrence of thermophilic fungi in aquatic sediment of lakes and rivers is mysterious in view of the low temperature (6–7 °C) and low level of oxygen (average 10 ppm, <1.0 ppm at a depth of 31 meters) available at the bottom of a lake. Undoubtedly, the thermophilic fungi owe their ubiquity and common occurrence, in large measure, to their ability to occupy a temperature niche that most other fungi are unable to inhabit. Thermophilic fungi are worldwide in distribution and most species do not show any geographical restriction. The tropical sites, however, favor recovery as a consequence of elevated temperature and more competitive microbial environment. They have been reported from a wide variety of habitats including different soil types and places where self-heating of plant material results in elevated temperatures. They have been isolated from natural as well as man-made habitats. Most significant natural habitat for saprophytic thermophilic fungi is the decomposing organic materials in which thermogenic conditions develop. Solar heat in the tropics and moisture content are the other factors that significantly influence the development of thermogenic conditions.

Thermophilic fungi are found in a variety of habitats from which they are easily isolated; therefore many investigators have been prompted to use their own strains in enzyme studies. Marked differences have been observed in properties of enzymes in strains from different geographical backgrounds and have raised doubts about correct identification and procedure used for enzyme

assays. A comparison of the properties of an enzyme from two or more strains by the same method will be necessary to determine if high variability is a characteristic feature of thermophilic fungi. If so, investigations of the mechanisms generating variation may provide opportunities for fundamental discoveries. It is therefore important that scientists share cultures of thermophilic fungi and cloned genes for better understanding and developing recombinant proteins. Several thermophilic fungi have been recovered from non-thermogenic environments like coal and moist soils in Australia, Antarctic and sub-Antarctic soils, aquatic sediments where bottom temperature never exceeds 6 or 7 °C. Thermophilic moulds such as *Chaetomium senegalense* and *Myceliophthora fergusii* have been isolated from temperate soils of North India. A limited survey of thermophilic fungi occurring in Indian environments has been carried out so far. The isolation of the thermophilic moulds from the unexplored regions of India is expected to yield novel thermophilic fungal strains which may be sources of useful biocatalysts and metabolites.

**Coprophilous Fungi:** The faecal matter of all mammals consists of intestinal bacteria, with herbivore faeces also containing a population of coprophilous fungi. Coprophilous fungi are adapted for nutrition, survival, reproduction and dispersal involving passage through the gut of an herbivore. Some coprophilous fungi are specific to particular types of animal, other seems to be generalists. There is often a distinct succession observable on decaying dung: some later colonizers may produce antibiotics to aid colonization. Various researchers including Webster (1970) and Wicklow (1992) have made significant contributions on the ecological diversity and comparisons of coprophilous fungal communities on different dung types. Filamentous moulds such as *Aspergillus leporis* (*A. flavus* group) were first isolated from white-tailed jackrabbit dung. Larger fungi such as *Coprinus plicatilis*, *C. comatus*, *C. cinereus*, etc. have adapted to dung substrates *Coprophilous* species include several species of *Ascobolus*, *Cheilymenia*, *Coprotus*, *Peziza*, *Saccobolus*, *Thecotheus*, *Pseudombrophila*, *Mycoarctium* and *Trichobolus* from Macaronesia.

Global interests in natural products have increased of late due to safety concerns of synthetic products. Consequently a large number of biologically active molecules are identified in many species of macro fungi throughout the world. Such bioactive mushroom metabolites are believed to have the capability to help in the revitalization of the immune system against a large number of pathogenic and non-pathogenic diseases. They are reported to serve as biological response modifiers with the capability to activate macrophages and T-cells, and to produce cytokines, interleukins and tumour necrosis factors.

Coprophilus macrofungi (*Coprinus. comatus*, *C. plicatilis*, and *C. cinereus*) are the producers of several bioactive metabolites (tri-sesquiterpenes, quinones, glucans, proteins, etc.) and enzymes (protease, phenoloxidases, etc.) with immune modulating, antifungal, antioxidant, thrombolytic, hypoglycemic and anti-protozoal effects and these metabolites can be



extracted and identified. Polysaccharides extracted from the mycelial culture of *C. cinereus* have been shown to contain anti tumour effects. Similarly, polysaccharides obtained from *C. comatus*, tested in mice, revealed hypolipidemic effects and antioxidant properties suggesting that their antioxidant activity could be directly or indirectly responsible for its hypoglycemic and hypolipidemic properties. Mwita *et al.* (2010) found that Tanzanian *C. cinereus* grown on dried grasses supplemented with cow dung manure exhibits activity against *Escherichia coli*, *Aspergillus niger* and *Candida albicans*. In view of the above attention be given to the study of Coprophilus fungi.

**Endophytic Fungi:** In recent times it has been realized that plants serve as a reservoir of untold numbers of organisms known as endophytes. By definition, these microorganisms (mostly fungi and bacteria) live in the intercellular spaces of plant tissues. Some of these endophytes may be producing bioactive substances that may be involved in a host-endophyte relationship. As a direct result of the role that these secondary metabolites may play in nature, they may ultimately be shown to have applicability in medicine. A worldwide scientific effort to isolate endophytes and study their natural products is now under way (Hyde and Soyong, 2008; Schulz *et al.*, 2002). It appears that all higher plants are hosts to one or more endophytic microbes. These microbes include the fungi, bacteria and actinomycetes, which primarily reside in the tissues beneath the epidermal cell layers, and the host tissues are transiently symptomless. Endophytes have recently been shown to be key elements in plant symbiosis, affecting plant community biodiversity, plant defense, plant growth, and host tolerance to stressful conditions. However, the diversity, geographic distribution, and host specificity of endophytes remain largely unknown (Arnold, 2007). Recent estimates of fungal diversity (Hawksworth, 2004) suggest that more than 90% of fungal species are not described, and there are no specific estimates of the number of existing endophytes (Selim *et al.*, 2014).

Geographical distribution of endophytes affects the diversity and productivity of host plants. In a comparative study it has been found that tropical endophytes not only provide more active natural products than temperate endophytes but they also produce more active natural products. This observation suggests the importance of the host plant in influencing the general metabolism of endophytic microbes (Strobel and Bryan, 2003). Screening for antimicrobial compounds from endophytes is a promising way to overcome the increasing threat of drug resistant strains of human and plant pathogens. Several antimicrobial metabolites isolated from endophytes belong to diverse structural classes, including, viz. alkaloids, peptides, steroids, terpenoids, phenols, quinones, and flavonoids. Many antimicrobial compounds were isolated from endophytes, which only occupied a small portion of total endophyte species; hence it is obvious that there is a great opportunity to find reliable and novel antimicrobial natural products in endophytes, which may be used as clinically effective antibiotics in future (Suryanarayanan *et al.*, 2012).

Endophytes are increasingly exploited as a source of potent anticancer and immunomodulatory agents. Around hundred anticancer compounds from different fungal species have been isolated and they belong to 19 different chemical classes with activity against 45 different cell lines. Several compounds like taxol, torreyanic acid, camptothecin and podophyllotoxin isolated from different endophytes have shown the anticancer activity. Isoprenylated chromone derivatives which were isolated from *Pestalotiopsis* sp., a fungal endophyte of *Camellia sinensis* have shown cytotoxic effect in breast cancer MCF-7 cell lines (Kharwar *et al.*, 2011).

There is emerging need for development of immunomodulatory agents in order to tackle the cancer development, autoimmune disease, transplantation, allergy and infectious diseases. Immunomodulatory drugs are mainly immunosuppressive and immunoregulatory molecules. Endophytes are already exploited for the production of immunomodulatory agents.

**Caterpillar fungus (*Cordyceps sinensis*):** Caterpillar fungus (*Cordyceps sinensis*) is an entomogenous ascomycete parasitizing various grass root-boring *Thitarode* caterpillars. Around 40 species of ghost moths have been found to be colonized by *Cordyceps*. After completing the process of infection by *Cordyceps* sp., the insect gets completely mummified and emptied of nutrients leaving exoskeleton filled with the fungal mycelium. During spring the slender, brown, club shaped fruit bodies emerge from the insect based ground reaching a height of 8-15cm. The reproduction of this fungus is dependent on a specific host. *Cordyceps* spp. are known to occur in Himalayas and few other places where altitude are between 3200-4800msl with 350-400mm annual precipitation. Such fungi need to be studied for their bioactive molecules. There is a need to conserve such fungi both in the field and in the lab as culturable fungi.

*Cordyceps* contains a number of compounds which are considered nutritional viz. vitamin E and K, essential amino acids, vitamin B1, B2 and B12, sugars, complex polysaccharides, proteins, sterols, nucleosides and trace-elements. *Cordyceps* spp. act as antioxidant, antitumour, anti-inflammatory and immunomodulatory, etc. Cordycepin is an antitumour, anti-bacterial and insecticidal agent. At present the market value being US \$ 20,000 per Kg and this has resulted in over exploitation of thus fungus and habitat sites. Therefore it needs both *in-situ* and *ex-situ* conservation by applying innovative methods.

**Fungal nanoparticles:** Nanotechnology is one of the fascinating and rapidly advancing sciences and possesses abilities to modernize technology. Nanoparticles can be used in crop disease management and nanoparticles are established to act upon pathogens as chemical pesticides. Nanoparticles are known to control bacterial blight of rice and some virus diseases. Khot *et al.* (2012) reported that great scope exists for nanotechnology to suppress plant pathogenic bacteria, viruses and fungi they have reported protection of the plant from pathogen and bringing down its population below economic threshold paving the way for nanopesticide formulation. Nanoparticles are also

helpful in the detection of phytopathogen and pesticide residue.

Synthesis of silver nano particle has been ubiquitous and such fungal species include that of *Trichoderma*, *Fusarium*, *Penicillium*, *Rhizoctonia*, *Pleurotus* and *Aspergillus*. Extracellular synthesis has been demonstrated by *Trichoderma* sp. and other fungi. *Fusarium oxysporum* has been used to synthesize Zirconia, Titanium, Cadmium sulfide and Cadmium serenade nanosize particles (Ahmad *et al.*, 2003).

#### CONTRIBUTIONS OF LATE DR. M. J. THIRUMALACHAR (1914-1999)

Dr. Thirumalachar had been an outstanding, brilliant and internationally reputed mycologist, microbiologist and biotechnologist. He has monographed rusts of India along with late Dr. B. B. Mundkur, doyen of Indian Mycology. He described new genera namely *Mundukurella*, *Narasimhania*, *Franzpetrakia*, *Georgefischeria* and *Sclerophthora*. His outstanding contributions include the culturing of non-culturable fungi like *Conidiobolus*, an entomogenous fungus besides describing lifecycle of *Ravenelia esculenta* (edible rust) and also rediscovered *Aecidium esculentum* from Maharashtra. In the field of antibiotics, his research contributions are well recognized which include the discovery of Hamycin, Dermostatin, Aureofungin and others which are therapeutic agents for curing human mycoses. Further discoveries include antamoebin from *Emericellopsis* sp.

The discovery of *Chainia*, a new actinomycete in 1955 laid the foundation for conducting research on antibiotics from actinomycetes. The biological world and in particular the mycological world has lost a brilliant, excellent, innovative, highly knowledgeable genius of mycological sciences and biotechnologist on 21<sup>st</sup> April, 1999. I salute him and generations will remember him for his worthy contributions.

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