

A Project Titled

Fungal Biomolecules
with Applications in
Agriculture and Pharmaceutics

A DISSERTATION REPORT

Submitted in partial fulfilment for the award of the
Degree of Masters of Science in Biochemistry

BY

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DECLARATION

I hereby declare that the matter presented in this dissertation entitled '**Fungal Biomolecules with Applications in Agriculture and Pharmaceuticals**' is based on the literature survey carried out by **Miss Bristi Baskar** in the School of Chemical Sciences, Goa University under the supervision of **Dr. Amrita Pradeep Kharangate**, and the same has not been submitted elsewhere for the award of degree.

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CERTIFICATE

This is to certify that the dissertation entitled '**Fungal Biomolecules with Applications in Agriculture and Pharmaceuticals**' is a Bonafide work carried out by **Miss Bristi Baskar** under my supervision in partial fulfilment of the requirements for the award of the degree of Masters of Science in Biochemistry at the School of Chemical Sciences, Goa University.

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INDEX

Serial No.	Title	Page No.
1.0	Abstract	1
2.0	Introduction	2
3.0	Fungal enzymes	6
4.0	Fungal antibiotics	10
5.0	Fungal plant growth promoters	12
6.0	Fungal siderophores	14
7.0	Fungal pigments	16
8.0	Fungal hormones	18
9.0	Fungal vitamins	22
10.0	Fungal organic acids	23
11.0	Conclusion	26
12.0	Bibliography	27

1.0 ABSTRACT

Fungi are a well-studied group of organisms with immeasurable potential for bioprospecting. Fungi have evolved by adopting different survival mechanisms as a result they can grow in a wide range of habitats where it is necessary to contend against a broad range of many other organisms such as bacteria and animals. The distinct properties of fungi hold significant potential for bioprospecting of fungi or its products in agriculture and pharmaceuticals. Fungi are easy to cultivate and care for, making the production of fungal biomolecules feasible. The massive search for biodiversity, as well as the creation of living fungus collections, has tremendous economic potential in terms of identifying species with unique industrial applications, which will lead to novel products. The importance of fungi in industries such as agriculture, pharmaceuticals, food, and feed is discussed in this paper. The study also highlights fungal biomolecules such as enzymes, antibiotics, organic acids, vitamins, hormones, siderophores, and plant growth-promoting fungi, as well as their uses in diverse sectors. Novel compounds such as penicillin, cephalosporin, alpha-amylase, lovastatin, as well as other commercially and globally valuable drugs have come from fungi. Despite this, fungi continue to be an under-utilized source with tremendous industrial and economic potential.

2.0 INTRODUCTION

When all organisms were classed as either animals or plants, fungi were initially classified as plants. Fungi were later classed as being part of a separate kingdom. Plants employ photosynthesis to produce nutrients, while animals get their nutrients by eating these plants and other animals. Fungi, on the other hand, receive nutrition by other means, such as the decomposition of plant and animal remnants, and are thus classified as saprophytes. As a result, fungi serve a crucial role in preserving the ecosystem's equilibrium. Fungi are eukaryotic creatures that produce mycelia, a basic filamentous structure, do not have photosynthetic capabilities, and have a cell wall made mostly of chitin and chitosan. The mode of reproduction in fungi is sporulation (Weber, 2007).

Since the first loaf of leavened bread was cooked and the first container of grape must was transformed into wine, humans have been exposed to fungi indirectly. Fungi can be found in vast quantities almost anywhere. Fungi, like bacteria, are responsible for decomposing organic matter and releasing carbon, oxygen, nitrogen, and phosphorus into the soil and the environment. The kingdom fungus has yielded some ground-breaking discoveries, which began in the nineteenth century and continued throughout the first half of the twentieth century (Cavalier-Smith et al., 2001). When Scottish bacteriologist Alexander Fleming spotted the green mold *Penicillium notatum* growing in a culture dish of *Staphylococcus* bacteria in 1928, he realized that fungi had medical significance. A transparent ring around the mouldy area in which no germs could grow (Dube, 2013). Fleming was able to extract a compound from mold that inhibited bacterial development. He announced the discovery of penicillin in a scientific study published in 1929, the first of a series of antibiotics—many of them derived from fungi—that have changed medical treatment. Some fungal species include compounds that are harvested and utilized to make medications like statins, which are used to lower cholesterol levels and prevent heart disease. Organic acids, enzymes, proteins, and vitamins are also made with the help of fungi (Ligon, 2004).

Many household and commercial operations, such as the manufacturing of bread, wine, cheese, and beer, require the presence of fungi. Because of their size, mushrooms were the only fungi seen in fields and forests before the discovery of the microscope in the 17th century. The invention of the microscope allowed for the recognition and identification of a wide range of fungal species. Fungi can be either terrestrial or aquatic, living in both

freshwater and marine habitats. Because they cannot survive high levels of salinity, freshwater species are usually found in clean, chilly water (Weber, 2007).

2.1 Basic structure and nutrition of a fungus

A fungus is made up of a mass of tubular filaments that are branched and contained in a rigid cell wall. These filaments are known as hyphae, and they branch radially to produce the mycelium, which is a growing network. The thallus, or undifferentiated body of the fungus, is formed by the mycelium. After reaching maturity, the mycelium produces fruiting forms called sporangium, which contain reproductive cells known as spores, by absorbing nutrients from dead and degraded materials. The spores are subsequently discharged into the environment and distributed by air and other environmental forces. The spores germinate and develop into hyphae that branch and produce mycelium of a new individual once they find a suitable substrate and favourable conditions. Some fungi, such as yeast, do not develop mycelium, but instead grow as single cells that proliferate through budding or, in some cases, fission (1971, Harley-Davidson).

Fungi grow on organic matter to meet their nutritional demands. They can digest and absorb a wide range of carbohydrates (glucose, sucrose, fructose, xylose, starch, cellulose, hemicellulose, and lignin). Proteins are also a source of carbon and nitrogen for many fungi. Fungi protect themselves from predators by secreting enzymes (biological catalysts) into the surface on which they grow. The meal is digested by the enzymes and absorbed directly through the hyphal walls (Magasanik, 1957).

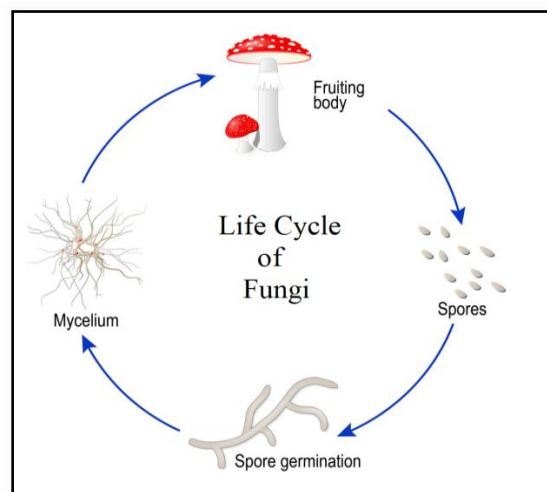


Fig. 1 Fungal Life Cycle

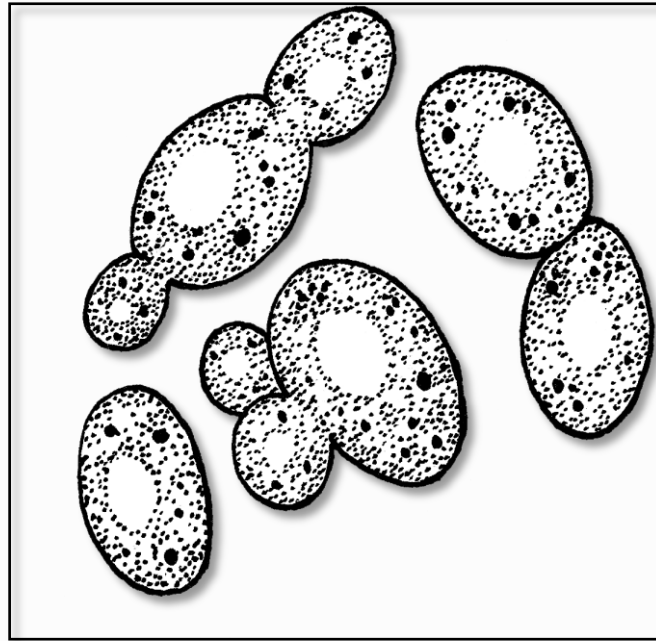


Fig. 2 Budding yeast cells

2.2 Bioprospecting of fungi

Fungi are a large range of eukaryotic organisms that are commonly employed for metabolites with potential medicinal benefit, as well as food and textile applications. This is due to the fact that fungi are a rich and diverse source of biomolecules that are being explored and developed for use in food, health, and the environment. Fungal biomolecules are a crucial tool for speeding up current research into the diverse functions of fungal biomolecules (Gupta, 2015).

Plant growth-promoting (PGP) fungi are becoming more popular as bio inoculants as a result of their multiple advantages in terms of plant quantity and quality, as well as their favourable interaction with the environment. Bacteria and mycorrhizal fungi make up the majority of PGP microorganisms found. (Verma et al., 2015a, b, c, 2016a) (Johansson et al. 2004; Kumar et al. 2018). Fungi have a number of traits that set them apart from bacteria. Fungi, for example, are significantly better at mobilizing bound phosphates and can endure acidic environments better than bacteria (Kumar et al. 2018; Wahid and Mehana 2000). In addition, fungi have been shown to create phytohormones such as indole-3-acetic acid (IAA), gibberellins (Kumar et al. 2018), and siderophores (Kumar et al. 2018; Milagres et al. 1999).

Microbe-derived enzymes have garnered widespread recognition for their wide range of applications in industries such as food, agriculture, chemical manufacturing, and medicine. In medicine, they are used to treat health problems caused by a lack of human enzymes as a result of genetic issues (Anbu et al. 2015; Singh et al. 2016a). Reduced process time, minimal energy input, cost-effectiveness, increased efficiency, nontoxicity, higher-quality products, and eco-friendly traits are all reasons why enzyme-mediated processes are becoming increasingly popular in the food, pharmaceutical, textile, paper, and leather industries. (Gurung et al. 2013; Kamini et al. 1999; Singh et al. 2016a). Due to the high and better performances of enzymes from various microorganisms that act under a wide variety of chemical and physical circumstances, industrial production of microbial enzymes is vital. Furthermore, microbial enzymes can be cultivated primarily through gene modifications, as required by companies (Gurung et al. 2013).

3.0 Fungal enzymes

Enzymes are proteins created by living organisms to catalyze metabolic processes. Every enzyme has its own use, and finds applications in the food industry, pulp and paper industry, textile industry, pharmaceutical industry, agriculture, biofuel production, and bioremediation. The enzymatic techniques have advantages over the traditional chemical approaches such as the combination of mild reaction conditions, increased selectivity and specificity of results in the process, fewer by-products and waste material. Therefore, it provides effective results, clean industrial processes, and contributes to the concept of long-term growth (De Souza et al. 2020).

3.1 Amylase

Amylases have been widely used in the food, textile, detergent, paper, and pharmaceutical sectors since the eighteenth century because of their ability to hydrolyze starch into simple sugars such as dextrans and oligosaccharides (Mehta and Satyanarayana 2016; Singh et al. 2016; Yadav et al. 2016a). α -Amylase is produced by fungi such as *Aspergillus oryzae*, *Aspergillus niger*, and *Aspergillus Terreus*, *Aspergillus awamori*, *Aspergillus. fumigatus*, *Aspergillus favus*, *Thermomyces lanuginosus*, *Paecilomyces variotii*, *Penicillium brunneum*, *Penicillium chrysogenum*, *Rhizopus oryzae*, *Mucor sp.* and *Trichoderma pseudo koningii* (Samanta, 2020).

Digestive medications contain α -Amylase from *Aspergillus oryzae* in syrup and pill form. *Aspergillus oryzae* is used to relieve dyspepsia. Due to its alkalophilic, chelator-resistant, and oxidant-insensitive nature, a detergent formulation incorporating α -amylases demonstrated potential utility in dishwashing (Samanta, 2020).

3.2 Glucose oxidase

Glucose oxidase is a type of enzyme that breaks down glucose. Glucose oxidase (GOD) uses atomic oxygen as an electron acceptor to catalyze the conversion of α -D-glucose into α -D-glucono-lactone. *Aspergillus niger*, *Aspergillus tubingensis*, *Aspergillus favus*, *Aspergillus terreus*, *Aspergillus oryzae*, *Aspergillus carbonarius*, *Aspergillus nidulans*, *Penicillium amagasakiense*, *Penicillium variable*, *Penicillium chrysogenum*, *Penicillium notatum*, *Penicillium funiculosum*, *Penicillium adametzii*, *Penicillium glaucum*, and *Penicillium vitale*, *Talaromyces favus*, *Phanerochaete chrysosporium*, *Alternaria alternata*, *Pleurotus ostreatus*,

Rhizopus stolonifer, and *Flavodon flavus* are used produce GOD at industrial scale (Kriaa and Kammoun 2016).

Calcium gluconate produced from GOD is used in the pharmaceutical industry to treat calcium deficiencies (Khurshid et al. 2013). The antibacterial and antifungal activities of GOD produced by *Aspergillus niger* and *Penicillium chrysogenum* are active against diverse fungal infections (Zia et al. 2013). Therefore the glucose oxidases from these fungal strains are commonly used to test blood glucose levels due to their potential usefulness even at extreme temperatures and pH (Wang and Lee, 2015).

3.3 Protease

The medical applications of protease enzymes include treatment for AIDS, cancer, and diabetes. *Aspergillus fumigatus*, *Aspergillus oryzae*, *Aspergillus versicolor*, *Aspergillus terreus*, *Aspergillus niger*, and *Aspergillus flavus* are the major fungal sources for protease enzyme (Yadav et al. 2017, 2020a, b). Proteases are employed in skin ointments for treating skin ulcers, removing keratin in acne, and degrading keratinized skin in the pharmaceutical industry (Shubha and Srinivas 2017; Devi et al. 2020a). Protease enzyme-based products that hydrolyze the peptide bonds of keratin and collagenolytic proteases have been used in wound healing and adenovirus-mediated cancer therapy in the cosmetic industry (Chao et al. 2007).

3.4 Cellulase

Cellulases are a type of enzyme that efficiently hydrolyzes polysaccharides and degrades cellulose chains found in biomass. Cellulases have been identified in the fungal species *Aspergillus fumigatus*, *Aspergillus niger*, *Myceliophthora thermophila*, *Rhizopus oryzae*, *Trichoderma reesei*, and *Thielavia terrestris* (Kupski et al. 2014; Kour et al. 2019c; Sahay et al. 2017; Yadav et al. 2019).

Cellulases are biocatalysts that can aid in the release of medicinal chemicals in the pharmaceutical business. Cellulases are digestive aids that are used to treat metabolic problems. Nondigestible fibres depolymerized by cellulases in a prebiotic increase the growth of beneficial bacteria in the intestine (Karmakar and Ray 2011).

3.5 Invertase

Invertase (β -fructofuranosidase) hydrolyzes sucrose, which means it breaks down the 1, 4-glycosidic bonds between D-glucose and D-fructose (Toledo et al. 2019). Some fungal species that produce invertase include *Aspergillus*, *Cladosporium*, *Fusarium*, *Aureobasidium*, *Trichoderma*, *Eremothecium*, *Ceratocystis*, *Metarhizium*, *Sclerotinia*, *Penicillium*, *Rhizopus*, and *Paecylomices*.

Fructooligosaccharides (FOS) produced by invertase is a nondigestible carbohydrate made up of fructose chains with a wide range of applications in the pharmaceutical business. Because of its decreased caloric value and short-chain fatty acids, FOS can be advised for diabetes individuals (Bhalla et al. 2017).

3.6 Lignase

Lignase is a heme protein that uses hydrogen peroxide to oxidize phenolic, non-phenolic, and aromatic chemicals (Vandana et al. 2018). It is a major enzyme in the breakdown of lignin and has a large catalytic region on the protein surface (Falade et al. 2017). White-rot fungus produces an enzyme called lignin peroxidase (LiP). *Phanerochaete chrysosporium*, *Trichoderma versicolor*, *Trichoderma viride*, *Aspergillus niger*, *Trichoderma reesei*, *Phlebia radiata*, *Trametes suaveolens*, and *Pleurotus ostreatus* are some of the lignin peroxidase-producing fungal species (Xu et al. 2013).

LiP-producing *Sporotrichum pruinosum* and *Ceriporiopsis sp.* can synthesize human skin melanin. Skin texture and roughness improved as a result of the ability to degrade human hair and skin melanin. In comparison to hydroquinone, the skin lightening effect of fungal LiP with no obvious side effects demonstrated the potential advantages in the cosmetic sector (Draelos, 2015).

3.7 Lipase

Lipases can hydrolyze insoluble triacylglycerols and catalyze reversal processes such as esterification, alcoholysis, and acidolysis. They also have high substrate specificity (Javed et al. 2018). *Aspergillus*, *Fusarium*, *Mucor*, *Geotrichum*, *Penicillium*, and *Rhizopus sp.* are the most prevalent fungal lipase producers. *Penicillium simplicissimum* has been researched for its ability to manufacture lipases in recent years.

Table no. 1: Fungal enzymes and their applications

Enzyme	Fungal source	Application	Reference
Amylase	<i>Aspergillus oryzae</i> <i>Penicillium chrysogenum</i>	Digestive medicines, Detergents	(Samanta, 2020)
Glucose oxidase	<i>Aspergillus niger</i> <i>Penicillium notatum</i>	Treatment of calcium-associated deficiencies, antibacterial, antifungal properties, biosensors	(Zia et al. 2013)
Protease	<i>Aspergillus fumigatus</i> <i>Aspergillus oryzae</i>	Treatment of AIDS, cancer, and diabetes, in skin ointments	(Shubha and Srinivas 2017)
Cellulase	<i>Rhizopus oryzae</i> <i>Aspergillus niger</i>	Biocatalysts in therapeutics, digestive medicines, prebiotics	(Karmakar and Ray 2011)
Invertase	<i>Aspergillus Fusarium</i> <i>Trichoderma sp.</i>	Digestive medicines, treatment of diabetes	(Bhalla et al. 2017)
Lignase	<i>White-rot fungi</i> <i>Aspergillus niger</i>	Synthesis of skin melanin, skin lightening	(Draelos, 2015)
Lipase	<i>Aspergillus Fusarium</i> <i>Penicillium chrysogenum</i>	Production of taxol-1, in biosensor	(Herrera-López 2012)
Chitinase	<i>Saccharomyces cerevisiae</i> <i>Trichoderma harzianum</i>	Antifungal activity, cancer treatment	(Gutiérrez-Román et al. 2015)

Penicillium chrysogenum HTF24, a psychrotrophic fungus that produces cold-active lipase, has various advantages in the industrial and environmental sectors (Rafiq et al. 2020). Lipase-mediated synthesis of Polixatel (taxol-1) was employed in the medical business to treat cancer cells (Fukaya et al. 2016). Triglycerides, cholesterol, and phospholipids levels in the blood can be detected using a lipase-based biosensor (Herrera-López 2012).

3.8 Chitinase

Fungal Chitinase hydrolyzes chitin and can be used to make single-cell proteins, N-acetyl-d-glucosamine, chito-oligosaccharides, and a variety of other medical products. *Saccharomyces cerevisiae*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Ustilago maydis*, *Coprinopsis cinerea*, *Trichoderma harzianum*, and *Xenorhabdus nematophila* are some of the chitinase-producing fungi (Liu et al. 2019; Rastegari et al. 2020a, b; Yadav et al. 2020e). Chitinase produced from fungi exhibits antifungal action and could be used in biocontrol (Le and Yang 2018). Antifungal medicines containing chitinase have shown promise in treating a variety of infections (Gutiérrez-Román et al. 2015). Fungi producing important enzymes have been mentioned in Table 1.

4.0 Fungal antibiotics

Because of the activity of soil organisms, soil is an extremely complex system with many constituents, each of which serves a particular role (Al-Enazi et al. 2018; Ullah et al. 2017). Microorganisms also play a crucial function in the soil ecology. Soil quality is determined by the microbial makeup and function. Fungi are extremely beneficial to the soil environment and play a vital part in human survival. Agriculture, bioremediation, natural cycles, the food business, and as bio-fertilizers fungi are essential (Karthikeyan et al. 2014; Yadav and Yadav 2018a). Secondary metabolites are also abundant among fungi. Because of the fierce battle for resources and territory in this microbially rich environment, soils have long been thought to be prolific reservoirs of antibiotic-producing bacteria. Over 60 years ago, soil organisms were used to screen antibiotics, and soil isolates have yielded a large percentage of known secondary metabolites from fungi. The synthesis of secondary metabolites such as antibacterial agents is one of the most important uses of fungi that can actually be beneficial for medical therapy (Al-Daamy et al. 2018; Farjana et al. 2014).

Secondary metabolites are small organic chemicals produced by an organism that are not essential for its growth, development, or reproduction, but are vital in antagonism, competition, and self-defense. These secondary metabolites protect the organism from other living species, allowing it to occupy the niche and consume the food. Fungi produce a variety of antibiotics with antibacterial and antifungal properties, including cephalosporin, fusidic acid, and penicillin, which are commonly used as medications (Al-Enazi et al. 2018).

Table no. 2: Fungal antibiotics and their applications

Antibiotics	Fungal source	Application	Reference
Penicillin	<i>Penicillium notatum</i> <i>Penicillium chrysogenum</i>	Bacteria	(Al-Daamy et al. 2018)
Pyrimidine A, B	<i>Verticillium sp.</i>	<i>Pyricularia oryzae</i>	(Higginbotham et al. 2013)
Altersolanol A	<i>Ampelomyces sp.</i>	<i>Staphylococcus aureus</i> , <i>Staphylococcus epidermis</i>	(Ribeiro et al. 2018)
Cephalosporin	<i>Cephalosporin acremonium</i>	Gram-positive bacteria	(Farjana et al. 2014)
Griseofulvin	<i>Penicillium nigricans</i> <i>Penicillium griseofulvum</i>	Certain fungi	(Saykhedkar and Singhal, 2004)
Fusidic acid	<i>Fusidium coccineum</i>	Bacteria	(Ribeiro et al. 2018)
Schizophyllan	<i>Schizophyllum</i>	<i>Staphylococcus aureus</i>	(Wiyakrutta et al. 2004)

Furthermore, vascular plant endophytes have been the most intensively studied ecological group in the last decade (Karwehl and Stadler 2016). Antibacterial, anticancer, antifungal,

anti-inflammatory, anti-malarial, and antiviral compounds have been found to be produced by fungal endophytes (Higginbotham et al. 2013; Supaphon et al. 2018; Wiyakrutta et al. 2004). Al-Daamy and others (2018) used the disc approach to screen the filtrates of *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Bacillomyces sp.*, *Cladosporium sp.*, *Penicillium notatum*, and *Trichoderma sp.* for antibacterial activity against *Bacillus sp.*, *Enterobacter sp.*, and *Klebsiella sp.* *Bacillus sp.* exhibited the highest sensitivity to all fungal filtrations in the investigation. *Trichoderma viridae* was isolated from cucumber rhizospheric soil and tested as an antibacterial, antioxidant, and anticancer agent by (Awad et al. 2018). *Trichoderma viride* inhibited the mycelial growth of *Fusarium solani*, *Rhizoctonia solani*, and *Sclerotium rolfsii*, according to the study. Furthermore, the alcoholic extract of the fungal mycelia showed significant antibacterial action against *Bacillus subtilis*, *E. coli*, and *Pseudomonas fluorescens*, as well as antifungal activity against *Candida albicans*, *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Pythium ultimum*. Extracts of *Curvularia sp.* and *Diaporthe sp.* inhibited pathogenic Gram-negative and Gram-positive bacteria, including *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, according to Ribeiro and others (2018).

5.0 Fungal plant growth promoters

Plant growth-promoting fungus (PGPF) and plant growth-promoting rhizobacteria (PGPR) are microorganisms that live in conjunction with plant roots and promote plant growth. These have been employed to induce resistance in host plants to invading phytopathogens, as well as to improve plant growth and development. The use of these microbes is an environmentally friendly disease control strategy that is long-lasting due to the activation of innate immunity in plants. Only a few studies comparing PGPF to PGPR have been published yet in order to determine its efficacy in inducing resistance to invading microorganisms. Non-pathogenic, naturally occurring saprophytes and plant growth-promoting fungi aid to maintain soil fertility which increases plant growth and activates the plant's first line of defense against pathogen infestations. The ability of PGPF to colonize roots is thought to be the first and most important mechanism in the avoidance of pathogen infection. It also aids in the intake of nutrients, which improves plant growth. Apart from promoting disease resistance, the ability of PGPF to solubilize phosphates, and produce Indole-3-acetic acid, siderophores and enzymes contribute directly or indirectly to the development and promotion of host plant growth (Naziya et al., 2019).

Researchers have focused their attention on the application of PGPF for the induction of resistance and promotion of plant growth by activating induced systemic resistance (ISR) in plants due to the substantial beneficial effects of PGPF in agriculture. In plants, ISR by PGPF entails the formation of lignin, cellulose, phenols, and other compounds in cell walls, which primarily block entry and also hinder the invading pathogen's growth and multiplication (Bulgarelli et al., 2013). Apart from modifying the plant cell wall, PGPF has been shown to increase the concentration of defense-related enzymes in plants (phenylalanine ammonia-lyase (PAL), peroxidases (POX), chitinase, glucose, and others) that are directly linked to phytopathogen defense mechanisms. There have been no reports of the use of PGPF in chilli for improved plant growth and disease resistance against anthracnose in the literature yet. As a result, a study was conducted to determine the efficacy of PGPF isolated from the chilli plant rhizosphere in the soil in promoting plant growth and inducing resistance to *C. capsicum* in chillies grown in greenhouses, as well as to elucidate the possible mechanism of action at the histological and biochemical levels (Timmusk et al., 2017).

Table no. 3: Plant growth-promoting fungi and their applications

PGPF	Application plant	Effect	Reference
<i>Penicillium sp.</i>	Sesame	Better root and shoot growth	Bulgarelli et al., 2013)
<i>Trichoderma harzianum</i>	Corn	Lessen oxidative stress	(Bissett, 1991)
<i>Exophiala sp</i>	Cucumber	Better growth under salinity	(Garca et al. 2005)
<i>Fusarium pallidoroseum</i>	Rice	Increased biomass production	(Samuels and Hebbar 2015)
<i>Trichoderma longibrachiatum</i>	Wheat	Better tolerance to salt stress	(Garcia-Garza et al. 1997)

Plants are protected from harmful microbes by PGPF, which induces systemic resistance (Hossain and colleagues 2017). Plant growth promotion, nutrient utilization, rhizosphere change, and suppression of phytopathogenic fungi via enhancing defense mechanisms have all been described in *Trichoderma spp.* (Garcia-Garza et al. 1997; Harman et al. 2004; Garca et al. 2005). A large range of bio fungicide agents and agro products based on *Trichoderma spp.* were listed by Verma and others (2007) and Samuels and Hebbar (2015). Similarly, Navi and Bandyopadhyay, (2002) mentioned commercially available biocontrol agents based on *Ampelomyces*, *Candida*, *Coniothyrium*, *Gliocladium*, *Talaromyces*, and other fungal plant pathogens. *Trichoderma species* are a natural and prospective benefit to farmers around the world because of their prevalence in a wide range of environmental conditions, ease of access, and broad scope of action (Ahmad and Baker, 1987). *Trichoderma sp.* was described by Bissett, (1991) as dendroidal branching conidiophores with phialides. *Trichoderma viride*, *Trichoderma atroviridae*, and *Trichoderma koningii* is divided into three divisions by Jash (2006): (1) *Trichoderma* (*T. viride*, *T. atroviridae*, and *T. koningii*), (2) *Pachybassium* (*T. virens*, *T. polysporum*, *T. harzianum*, and *T. piluliferum*), (3) *Longibrachiatum* (*T. aureoviridae* and *Gliocladium viride*). Fungi producing plant growth-promoting hormones have been listed in Table 3.

6.0 Fungal Siderophores

In reaction to limited iron availability in the environment, bacteria and fungus produce siderophores, which are microbial, iron-chelating molecules. Nearly all microbes create siderophores, which are low molecular weight iron-chelating ligands. Iron uptake by siderophores is a complex process that changes depending on the type of iron-chelating molecule. Phenolates, hydroxamates, and polycarboxylates are the three most prevalent classes found. Fungi produce a variety of hydroxamate siderophores. High-affinity ferric iron reductase and siderophore productions are two important reactions to iron stress in fungus. Some phytopathogenic fungi produce biochemicals that are phytotoxins as well as iron chelators (Byers and Arceneaux 1998; Haselwandter and Winkelmann 2002; Fleischmann and Lehrer 1985).

In medicine, siderophores are used to treat iron and aluminium overload, as well as to improve antibiotic targeting. Because of their strong affinity for iron, they are valuable as medications for aiding iron mobilization in humans, particularly in the treatment of iron

disorders. By producing conjugates between siderophores and antimicrobial medicines, the transportability of siderophores is employed to transport medications into cells. Because only particular siderophores are recognized and utilized by bacteria, such conjugates are expected to have selective antimicrobial activity. Cefiderocol, a cephalosporin antibiotic, is an example. Siderophores are recognized as iron delivery agents in siderophore-mediated drug delivery, allowing the microorganism to absorb siderophore conjugates with attached medicines. When the bacterium assimilates the siderophore conjugate, these medications are extremely deadly to it, causing apoptosis. The iron-binding functional groups of siderophores have been added to antibiotics, considerably increasing their efficacy (Wencewicz et al., 2009).

Agriculturally essential crops like barley and wheat can efficiently sequester iron by releasing phytosiderophores into the soil rhizosphere via their roots. Chemical compounds produced by rhizosphere microorganisms can help improve iron availability and uptake. Natural and manmade siderophores can bind metal ions other than iron ions. Aluminium, gallium, chromium, copper, zinc, lead, manganese, cadmium, vanadium, zirconium, indium, plutonium, berkelium, californium, and uranium are some examples of rare earth elements (Ahmed & Holmström, 2014).

The major siderophores released by ericoid mycorrhizal fungus are ferricrocin or fusigen. The ectomycorrhizal fungus *Cenococcum geophilum* and *Hebeloma crustuliniforme* have also been shown to produce ferricrocin. The hydroxamate siderophores coprogens are produced by *Trichoderma spp.* and *Fusarium spp.* The siderophores produced by *Aspergillus niger*, *Penicillium citrinum*, and *Trichoderma harzianum* boost the shoot and root lengths of chickpeas, according to a study on the plant-growth-promoting properties of fungus (Yadav et al. 2011).

Fungal siderophores have a broad range of shapes and colors. Hydroxamate and carboxylate siderophores are produced by fungi and have been researched mainly in *Aspergillus* species. For example, *Aspergillus fumigatus* and *Aspergillus nidulans* produce around 55 different forms of siderophores. These species are saprotrophs, meaning they contribute to the environment's carbon and nitrogen cycles. Many fungi may synthesize multiple siderophore types, especially when the iron is scarce. To tap external iron, *Aspergillus fumigatus* produces a hydroxamate siderophore and triacetyl fusarine C (TAFC). This fungus can also secrete ferricrocin, a siderophore that helps in hyphal iron mobilization and dispersion, as

well as intracellular iron storage. *Aspergillus fumigatus* also produces the conidial siderophore 'hydroxy ferricrocin' to store iron in its conidia and minimize oxidative stress during the germination phase. Ferricrocin (Frr) and ferrihordin are the two primary siderophores produced by *Aspergillus nidulans*. Fusigen (Fsg) is an unacetylated version of TAFC generated by a twenty-four-hour culture of *A. nidulans*. Due to the degradation and uptake of fusigen, an older strain (48 h) produced acetylated TAFC. A fungus identified as a brown-rot fungus, *Wolfiporia cocos*, may produce distinct forms of catecholate siderophores. Apart from hydroxamates, the two common ectomycorrhizal basidiomycetes *Laccaria laccata* and *Laccaria bicolor* can create linear ester-containing Fsg siderophores, such as CPG, Frr, and TAFC (Haselwandter et al.1992; Pecoraro and colleagues, 2021).

7.0 Fungal pigments

Synthetic colorants have negative effects associated with health. Therefore, research has shifted to the discovery of natural pigments, which are becoming increasingly important around the world. Over the last decade, many natural pigments have been identified from a variety of sources, including plants, animals, insects, and microorganisms. The colors produced by microorganisms are of particular interest (Dufosse, 2006). Fungi, among other microorganisms, play an important role in the production of safe pigments; additionally, fungi have been observed to produce greater quantities of color (Kirti et al. 2014). Fungi produce a variety of primary and secondary metabolites, organic acids, enzymes, pigments, and other food additives, immune modulators, anticancer, antioxidants, and antiproliferative biomolecules that have medicinal applications. Furthermore, natural colorants have antibacterial properties, and have less likelihood of being allergic. They are also environmentally friendly and more stable than synthetic colorants (Velmurugan et al. 2010). Fungal pigments are secondary metabolites that are occasionally produced as a result of nutritional constraints. When the availability of critical nutrients is depleted or when the environment is unfavorable, mycelium creates secondary metabolites (Gupta and Aggarwal, 2014). Some fungi, such as *Aspergillus niger*, *Fusarium niger*, *Penicillium niger*, and *Trichoderma niger*, produce pigments as intermediate metabolites throughout their growth (Table 5). Carotenoids and polyketides are two types of fungal pigments. Tetraketides and octaketides with eight C₂ units create the polyketide chain in fungal polyketides (Atalla et al. 2011).

Table no. 5: Fungal pigments and their applications

Pigments	Fungal source	Colour	Application	Reference
Ankaflavin	<i>Monascus sp.</i>	Yellow	Reduce tumor neuron factor (TNS)	(Dufossé et al., 2005)
Rubropuntatin	<i>Monascus sp.</i>	Orange	Drug delivery	(Hsu et al., 2011)
Riboflavin	<i>Ashbya gossypii</i>	Yellow	Dietary supplement	(Dharmaraj et al., 2009)
Naphthoquinone	<i>Cordyceps unilateralis</i>	Dark red	Dietary supplement	(Gupta and Aggarwal, 2014)
Monasco rubin	<i>Monascus sp.</i>	Red	Food preservative	(Andersen et al., 1991)
Anthraquinone	<i>Penicillium oxalicum</i>	Red	Laxative, antimicrobial, anti-inflammatory	(Andersen et al., 1991)
Lycopene	<i>Blakeslea trispora</i>	Red	Dietary supplement	(Mata-Gómez et al., 2014)
β – carotene	<i>Blakeslea trispora</i>	Yellow	Dietary supplement	(Mata-Gómez et al., 2014)
Melanin	<i>Aspergillus fumigatus</i>	brown	Cosmetics	(Hyde et al., 2010)

8.0 Fungal hormones

Many fungi interact with plants in advantageous ways, for example, mycorrhizal symbiosis, or in detrimental ways, such as fungal infections (Sanders, 2011; Dean et al., 2012). Both symbiotic and pathogenic fungi penetrate their hosts without disrupting the plant cell plasma membrane in order to get nutrients. Fungi have developed mechanisms that include protein effectors and metabolites to counteract plant immunity and/or create favorable conditions for their invasion of plant tissues (Kamoun, 2007). Fungi generate chemicals that are analogous to plant hormones, such as auxins, cytokinins (CKs), gibberellic acids (GAs), ethylene (ET), abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA), in addition to conventional effectors. These hormones are well-known for controlling plant development and initiating crucial plant signaling events in response to biotic and abiotic stressors (De Vleeschauwer et al., 2013; Peleg and Blumwald, 2011; Pozo et al., 2015; Robert-Seilaniantz et al., 2011; Spence and Bais, 2015). The first study that implicated the pathogenicity of fungal-derived hormones was in gall-forming infections (Denancé et al., 2013; Robert-Seilaniantz et al., 2007).

8.1 Auxins

Auxins are indole-derived hormones that play a role in plant development, such as cell division, differentiation, and organ formation, as well as senescence (Benjamins and Scheres, 2008; Oka et al., 1999; Vanneste, 2005; Kim et al., 2011). Auxins influence plant biotic and abiotic stress responses. (Peleg and Blumwald, 2011). Auxins are produced and secreted by a wide range of fungal taxa, not just plant-interacting fungi, suggesting that these hormones may play an endogenous role in these organisms. Auxins are produced by *Fusarium sp.* and *Gloeosporioides sp.* (Gruen, 1959; Ulrich, 1960). Auxins have a role in symbiotic relationships between bacteria and fungi. They are necessary for the production of nodules in the nitrogen-fixing bacterial symbiosis and the invasion of mycorrhizal fungi (Hirsch and Fang, 1994; Etemadi et al., 2014; Hanlon and Coenen, 2011). For example, mutants of the ectomycorrhizal species *Hebeloma cylindrosporum* that overproduce auxin were able to enter root tissues of *Pinus pinaster* with greater ease indicating the auxins play a crucial role in plant tissue invasion (Gay et al., 1994; Laurans et al., 2001).

8.2 Cytokinins (CK)

Plant hormones generated from adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate or the tRNA breakdown route are known as CKs. CKs are well-known for their involvement in plant growth, such as root and shoot production, through the cell cycle and cell differentiation control. Cytokinins are produced by *Mucor Oryzae* (Barciszewski et al., 1999; Fosket and Torrey, 1969; Riou-Khamlichi et al., 1999).

8.3 Gibberellic acid (GA)

Gibberella fujikuroi produces terpenoid hormonal chemicals, which have been isolated for the first time. This fungus causes the rice disease 'bakanae,' or 'foolish seedlings,' in which afflicted plants grow unnaturally tall. The role of GAs in plant physiology was investigated after this discovery. Germination, blooming, cell division, and internode elongation are all controlled by GAs (Brian and Elson, 1954; Pimenta Lange and Lange, 2006; Swain and Singh, 2005). GAs has been found to boost the growth of immature hyphae of the ascomycete fungi *Neurospora crassa* in liquid culture and to increase conidial germination (Nakamura et al., 1978; Tomita et al., 1984). These effects are additive to auxin actions, implying that these two hormones may alter *Neurospora* germination and growth independently (Tomita et al., 1984).

8.4 Abscisic acid (ABA)

ABA is well known in plants for inducing stomatal closure and hence contributing to drought tolerance (Beardsell and Cohen, 1975). Peleg and Blumwald, (2011) identified ABA as the primary hormone for plant abiotic stress responses, that plays a role in seed dormancy by inhibiting the GA pathway (Debeaujon and Koornneef, 2000). It is considered that the mevalonate pathway is mostly engaged in fungi and that many ABA precursors can be utilized (Morrison et al., 2015b; Oritani and Kiyota, 2003). *Cercospora risicola* was the first fungus to produce ABA (Norman et al., 1983). Many fungi with various lifestyles (saprophytic, symbiotic, and pathogenic) have been identified as producing ABA since then (Crocoli et al., 1991; Esch et al., 1994; Jiang et al., 2010; Morrison et al., 2015b). Only two reports of ABA altering mycelium growth have been found. Exogenous administration of ABA to *Ceratocystis fimbriata* resulted in a modest increase in fungal growth. ABA boosted germination and the production of appressoria in *Mucor oryzae*, a specialized infection structure that breaks down the plant cell wall and allows invasion (Spence and Bais, 2015).

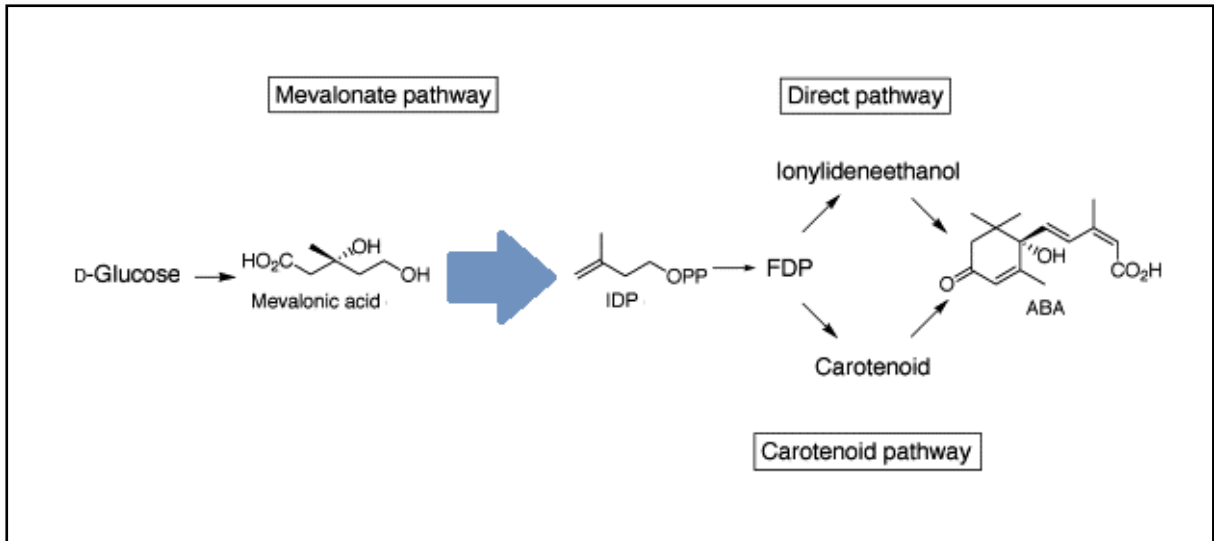


Fig. 3 Biosynthesis of abscisic acid via ionylideneethanol

IDP- isopentenyl pyrophosphate, FDP- farnesyl diphosphate, ABA- abscisic acid

8.5 Ethylene (ET)

ET is a gaseous molecule produced by *Penicillium digitatum* that was first recognized to play a role in the maturity of fruits (Bleecker and Kende, 2000; Payton et al., 1996). ET was later discovered to be involved in senescence, germination, flowering, and root and shoot development suppression (Bleecker and Kende, 2000; Grbic and Bleecker, 1995). The role of ET in mycorrhiza is determined by the type of symbiotic relationship. Mycorrhizal roots had a low amount of ET (McArthur and Knowles, 1992), and exogenous ET reduced AM growth (Geil et al., 2001; Zsögön et al., 2008). As a result, it has been proposed that AM fungi need to suppress the ET route in order for symbiosis to take place.

8.6 Salicylic acid (SA), Jasmonic acid (JA)

In most plants, SA and JA elicit antagonistic defenses against fungal biotrophic and necrotrophic diseases, respectively (Bari and Jones, 2009; Robert-Seilaniantz et al., 2011). Some fungal diseases create a single hormone to block the defense route that is most harmful to their proliferation. Pathogenic fungi such as *Gibberella fujikuroi* and *Botryodiplodia theobromae* have been found to produce JA in several studies (Miersch et al., 1991, 1992).

Table no.6: Fungal hormones and their applications

Hormone	Fungal source	Application	Reference
Auxins	<i>Fusarium sp.</i> <i>Gloeosporioides</i>	Nodule initiation in plants, control stress	(Gruen, 1959)
Cytokinins	<i>Mucor oryzae</i>	Plant development	(Barciszewski et al., 1999)
Gibberellic acid	<i>Gibberella fujikuroi</i>	Control germination, flowering, cell division, internode elongation	(Brian and Elson, 1954)
Abscisic acid	<i>Cereospora risicola</i>	Induce stomatal closure	(Norman et al., 1983)
Ethylene	<i>Penicillium digitatum</i>	Fruit maturation	(Bleecker and Kende, 2000)
Salicylic acid	<i>Ustilago maydis</i>	Defense	(Bari and Jones, 2009)
Jasmonic acid	<i>Gibberella fujikuroi</i>	defence	(Miersch et al., 1991, 1992)

JAs are oxylipins, as they are produced by lipid peroxidation. Some oxylipin production routes in fungal cells have been discovered and characterized. Both the host and the fungus physiological processes are affected by JA and the other oxylipins (Tsitsigiannis and Keller, 2007). In vitro treatment of methyl jasmonate to *Fusarium oxysporum* inhibited spore

germination and mycelium growth. *Mucor oryzae* has recently been discovered to influence JA homeostasis by generating JA derivatives and secreting a monooxygenase that converts rice endogenous JA to hydroxylated JA (12OH-JA) (Król et al., 2015).

9.0 Fungal vitamins

Vitamins are complex chemical molecules that are necessary in trace amounts for an organism's regular activities. Mammals, on the other hand, cannot synthesize many vitamins on their own and must rely on dietary supplements and feed additives to get them. Large-scale manufacturing of vitamins by microbes has been carried out over the last few decades, and more than half of commercially generated vitamins are fed to domestic animals (Table 7)(Ledesma-Amaro et al., 2015; Lee, 2015).

Table no.7: Fungal vitamins and their applications

Vitamin	Fungal source	Application	Reference
Vitamin B12	<i>Ashbya gossypii</i> <i>Candida sp.</i>	Pharmaceuticals (energy, immune system, hair, skin, nails, etc.)	(Nout & Rombouts, 1990)
Vitamin B5	<i>Fusarium oxysporum</i>	Pharmaceutical (Rheumatoid arthritis, osteoarthritis, PMS, Parkinson's disease)	(Mugula, 1992)
Vitamin B3	<i>Candida boidinii</i>	Pharmaceuticals (cholesterol, Parkinson's disease, blood pressure)	(Diplock et al., 1961; Fleet, 1990)

The tempeh fungus *Rhizopus oligosporus* can create a variety of vitamins, including niacin (B3), riboflavin (B2), pyridoxine (B6), pantothenic acid, and thiamine (Nout & Rombouts, 1990; Mugula, 1992; Shurtleff & Aoyagi, 2001; Nout & Kiers, 2005). *Rhizopus oryzae*,

another major fungus discovered in tempeh products (Samson et al., 1987), has been shown to generate niacin, vitamin K, ergosterol, tocopherol, as well as pyridoxine, riboflavin, and biotin (Mugula, 1992; Wiesel et al., 1997).

Because *Rhizopus oryzae* mycelium is less dense, it cannot be used to make tempeh on its own (Sharma & Sarbhoy, 1984). When tempeh was co-inoculated with *Rhizopus oligosporus*, however, the vitamin content increased (Mugula, 1992; Wiesel et al., 1997). It is hypothesized that *Rhizopus oryzae* may use α -galactosidase as a carbon source; hence it develops quicker in mixed culture than *Rhizopus oligosporus*. To avoid *Rhizopus oryzae* overgrowth in mixed-culture fermentation, *Rhizopus oligosporus* is injected at a higher level than *Rhizopus oryzae* (Wiesel et al., 1997). Due to amylase activity and lactic acid generation from glucose, *Rhizopus oryzae* is linked to sour off-flavors in tempeh, especially if the tempeh is prepared from starch-containing source materials (Hesseltine et al., 1985). Finally, *Rhizopus oryzae* may not be suited for the development of barley tempeh.

Yeasts can also make a variety of vitamins. The inclusion of carefully chosen yeasts is likely to boost the nutritional content of tempeh. Adding *Saccharomyces cerevisiae* to the barley tempeh fermentation raised the vitamin B6 and niacinamide levels (Diplock et al., 1961; Fleet, 1990).

10.0 Fungal organic acids

Although living cells synthesize many organic acids, only a few are commercially manufactured. Large-scale bio-processes are used to produce citric, gluconic, itaconic, and lactic acids. Fungal bioprocesses can produce oxalic, fumaric, and malic acids, but market demand is low because rival chemical conversion techniques are currently more cost-effective. A few different organic acids have been studied in the hopes of developing new methods. Citric acid and gluconic acid, both produced by *Aspergillus niger* fermentation of glucose or sucrose, have the biggest commercial quantities of fungal organic acids to date. *Aspergillus niger* is another *Aspergillus* species. Itaconic acid is made from the *Aspergillus terreus*. These three fungi were initially chosen for process development because they were capable of producing substantial volumes of a certain organic acid (Magnuson & Lasure, 2004). Many strains of *Aspergillus niger* can reduce the pH of their surroundings by oxidizing glucose outside the cell wall and converting it to glucose oxidase. Catabolism of

gluconic acid is rarer than glucose catabolism, and gluconic acid is also a powerful chelator and acidulant. Other *Aspergillus niger* strains make citric acid intracellularly and export it, possibly as a chelator and acidifier that can be reabsorbed and used as a carbon source. Itaconic acid produced by *Aspergillus terreus* acidifies the environment. Because itaconic acid is not a major metabolite, its anabolism and catabolism are rather uncommon metabolic characteristics. The use of itaconic acid to acidify the environment will restrict the growth of numerous microbes once again. As a result, due to the peculiar nature of itaconic acid, only *Aspergillus terreus* and a few other species would be able to catabolize it (Sahasrabudhe & Sankpal, 2001).

. Table no.8: Fungal organic acids and their applications

Organic acid	Fungal source	Application	Reference
Fumaric acid	<i>Rhizopus oryzae</i> <i>Rhizopus formosa</i>	Pharmaceutical Food industry	(Liaud et al., 2014)
Citric acid	<i>Aspergillus niger</i> <i>Aspergillus flavus</i>	Food industry Pharmaceutical	(Magnuson & Lasure, 2004)
Itaconic acid	<i>Saccharomyces cerevisiae</i>	Absorbents	(Sahasrabudhe & Sankpal, 2001)
Gluconic acid	<i>Aspergillus niger</i>	Food industry	(Liaud et al., 2014)
Kojic acid	<i>Aspergillus oryzae</i>	Food industry Cosmetic	(Magnuson & Lasure, 2004)

It's worth noting that *Aspergilli* and all other filamentous fungi in the Ascomycota phylum don't create lactic acid. The phylum Zygomycota appears to be the only one capable of producing huge volumes of lactic acid. Perhaps fungus categorized as Zygomycetes, such as *Rhizopus oryzae*, has evolved a new technique for acidifying the environment by creating lactic acid in order to compete with fungi that can't metabolize it. Many of these fungi produce both ethanol and lactic acid, a combination that would put many competitors to sleep. These four fungi-produced commercial organic acids are used in high-volume, low-value applications. They're used in industrial metal cleaning and other metal treatments, as well as taste enhancers, acidifiers, stabilizers, and preservatives in the food and feed business (Magnuson & Lasure, 2004). Although living cells synthesize many organic acids, only a few are commercially manufactured. Large-scale bio-processes are used to produce citric, gluconic, itaconic, and lactic acids. Fungal bioprocesses can produce oxalic, fumaric, and malic acids, but market demand is low because rival chemical conversion techniques are currently more cost-effective. A few different organic acids have been studied in the hopes of developing new methods (Liaud et al., 2014).

11.0 CONCLUSION

Fungi are an incredibly diverse group that can both benefit and harm humans. They play a significant role in the recycling of organic matter in the environment. Many antibiotics, organic acids, industrial alcohol, enzymes, and other products have been derived from fungi since the discovery of penicillin. Furthermore, fungi are used in the food industry to produce a wide variety of key items such as bread, alcohol, dairy products, meat, and sauces. Fungal enzymes have been investigated for catalysis in the food and feed industries to extend shelf life and improve nutritive value and yields, in addition to their role as catalysts in decomposing biomass and waste. Despite the fact that biomolecules are found everywhere, some species create biomolecules, particularly secondary metabolites, for highly specialized purposes during development and pathogenicity, or to preserve ecological interactions. Several fungi can benefit plants by improving nutrient absorption, shoot biomass, photosynthesis, and yield through improved root branching capacity. Fungal enzymes are used in agronomy to improve soil quality and crop productivity. The ability of fungi to produce plant growth-promoting hormones and confer disease resistance to plants has been extremely useful in agriculture. Therefore, bioprospecting of fungi by using advanced techniques will make it easier to find intriguing fungal biomolecules with potential applications in both agriculture and pharmaceuticals.

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