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REVIEW ARTICLE

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A Comprehensive Review of the Diversity of Fungal Secondary Metabolites and Their Emerging Applications in Healthcare and Environment

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ABSTRACT

Fungi and their natural products, like secondary metabolites, have gained a huge demand in the last decade due to their increasing applications in healthcare, environmental cleanup, and biotechnology-based industries. The fungi produce these secondary metabolites (SMs) during the different phases of their growth, which are categorized into terpenoids, alkaloids, polyketides, and non-ribosomal peptides. These SMs exhibit significant biological activity, which contributes to the formulation of novel pharmaceuticals, biopesticides, and environmental bioremediation agents. Nowadays, these fungal-derived SMs are widely used in food and beverages, for fermentation, preservatives, protein sources, and in dairy industries. In healthcare, it is being used as an antimicrobial, anticancer, anti-inflammatory, and immunosuppressive drug. The usage of modern tools of biotechnology can achieve an increase in demand for these SMs and large-scale production. The present review comprehensively analyses the diversity of fungal SMs along with their emerging applications in healthcare, agriculture, environmental sustainability, and nutraceuticals. Here, the authors have reviewed the recent advancements in genetic engineering, metabolic pathway manipulation, and synthetic biology to improve the production and yield of these SMs. Advancement in fermentation techniques, bioprocessing, and co-cultivation approaches for large-scale production of SMs. Investigators further highlighted the importance of omics technologies in understanding the regulation and biosynthesis of SMs, which offers an understanding of novel applications in drug discovery and sustainable agriculture. Finally, the authors have addressed the potential for genetic manipulation and biotechnological innovations for further exploitation of fungal SMs for commercial and environmental benefits.

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1. Introduction

Fungi and humans have a long history together, especially in terms of their chemical interactions. This strong connection was depicted by Alexander Flemming in 1928, who observed the killing of the bacterium by a mold *Penicillium notatum* on a petri dish inoculated with *Staphylococcus aureus* [1]. Fungi, a diverse group of organisms, have developed several communication and protection strategies, one of which involves the synthesis of a diverse group of bioactive compounds [2], also known as secondary metabolites (SMs) [3]. These SMs are important for the fungi for the interaction with the environment and strategies for survival but are not required for the reproduction of the fungi. The fungi have played an important role in shaping human society, which is evident from the discovery of penicillin in 1944 [4]. Due to the continuous investigations on fungal SMs, today, several life-saving drugs are available in the market.

Fungal SMs consist of a wide range of compounds, like alkaloids, terpenoids, polyketides, and non-ribosomal peptides (NRPs) [4]. The production

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of these SMs is from the metabolic pathways in which acetyl-CoA is the initial component in the production of terpenes (carotenoids) and polyketides (aflatoxins) [5]. Recently, fungal SMs have gained huge importance due to their intricate structure and diverse biological activity (antibacterial, immunosuppressive, and anticancer). These fungal SMs are formed at different growth phases or in response to environmental triggers, which fulfill various ecological roles like defense mechanisms, interspecies communication, and resource competition. The structural variety and biological properties of these SMs make them highly significant in the fields of pharmaceutical research, health, environmental sustainability, agriculture (as biopesticides and plant growth promoters), and industrial applications.

Within the natural environment, fungal SMs have a significant role in influencing microbial populations and the interactions between plants and fungi. Some of the fungal SM, have antibacterial [6], antifungal, and herbicidal properties, due to which fungi could thrive in the competitive environment. Recently, these fungal SMs have been widely used in the field of biotechnology for the advancement of innovative drugs, like antibiotics, immunosuppressants, and anticancer drugs [4]. In the whole world, a large number of studies are going on for the development of novel drugs from fungal SMs.

A team led by Zhang examined 174 species of *Aspergillus* and found a substantial presence and variety of SM backbone genes throughout the genus. Further, the investigator observed an increase in diversity among different sections. The localization of biosynthetic gene clusters (BGCs) in *Aspergillus* species exhibited a pronounced inclination toward low-synteny regions, with a distinct and notably biassed localization in sub-telomeric regions. This localization was found to impact gene expression variability, likely influenced by specific histone post-translational modifications (PTMs) such as H3K4me3 and H3K36me3.

Song et al. identified a total of 112 SMs from Microsphaeropsis fungi, including polyketones, macrolides, terpenoids, and N-containing compounds. Among the compounds discovered, 49 were novel, showcasing the significant contribution of Microsphaeropsis to the field of pharmaceutical research through the production of structurally unique and biologically active compounds [7].

The present review article emphasized the harnessing of the secondary metabolites of the fungi. The authors further reviewed the biosynthesis pathway, industrial production, and emerging techniques for the production of fungal secondary metabolites. Further emphasis has been given to genetic manipulation at the molecular level to increase the yield. Finally, the investigations have highlighted the applications of secondary metabolites in the environment, agriculture, biomedicine, biofuels, energy, etc. Such a study focuses on the greener routes for the production of secondary metabolites and their possible eco-friendly applications.

2. Biosynthesis and production of fungal secondary metabolites

It is very important to understand the biosynthesis and regulatory mechanisms of SMs, which is essential for exploring their potential in pharmaceuticals, agriculture, and biotechnology [8]. SMs are usually produced by fungi late in the growth cycle or during the idiophase since they are not very important during the exponential phase of growth. The synthesis of these metabolites depends upon the biochemical capabilities of the fungi and environmental stimuli that activate the expression of BGCs, which are DNA segments that are responsible for the formation of a set of proteins, primarily SMs [9]. It has been reported that not all metabolites are produced under standard culture conditions. To stimulate BGCs, it is necessary to manipulate the culture conditions and composition of growth media that will further stimulate a cellular response, leading to SMs production. However, BGCs of the desired product can be heterologously expressed in hosts, such as yeast, but these methods require molecular engineering tools to manipulate the genome of a particular species. Alteration of chromatin structure by deletion of histone deacetylases is an example of genetic modifications to trigger the activation of SM pathways. One of the most used methods to induce the synthesis of these metabolites is to subject fungi to stress conditions, such as oxidative or osmotic stress that will stimulate the activation of molecular defensive pathways leading to SMs production. For instance, the biosynthesis of penicillin from *Penicillium chrysogenum* initiates when the fungus starts consuming lactose after the glucose molecule has been depleted from the culture medium [10].

Singh et al. reported the production of SMs from *Fusarium oxysporum*, which is an endophytic fungus (EF). Further investigation revealed the potential of the extracted SM in the fields of anti-cancer, anti-malarial, anti-tuberculosis, antiviral, and anti-inflammatory. The isolated metabolites were characterized and confirmed by electrospray ionization mass spectrometry (ESI-MS), tandem mass spectrometry (MS-MS), and nuclear magnetic

Table 1. Key pathways in fungal secondary metabolite production.

Pathway type	Description	Key enzymes/genes involved	References
Polyketide pathway	Produces polyketides, a diverse group of compounds with antibiotic properties	PKS enzymes, often found in BGCs	[15,16]
NRP pathway	Synthesizes NRPs, which include important antibiotics and toxins	NRPs synthetase enzymes are also organized in BGCs	[15,16]
Terpenoid pathway	Responsible for the production of terpenes, which have diverse biological activities	Terpene synthase enzymes, often encoded by clustered genes	[17]
Alkaloid pathway	Produces alkaloids, known for their pharmacological effects	Various enzymes, depending on the specific alkaloid, often regulated by transcription factors	[11,17]
Hybrid pathways	Involves the combination of different biosynthetic routes, leading to complex molecules	Enzymes from multiple pathways, such as PKS-NRPS hybrids	[18]

resonance (NMR) and found Vinblastine and Vincristine as the important metabolites from *F. oxysporum* [11].

2.1. Pathways involved in secondary metabolite production

SMs from fungi could be synthesized by using several enzymatic pathways, but the most prominent ones are the polyketide, non-ribosomal-peptide (NRPs), terpenoid, and alkaloid pathways. In addition to all these, there is an alternative pathway known as a hybrid pathway [9]. Polyketides are conjugated by polyketide synthases (PKSs), multi-domain enzymes that catalyze the sequential condensation of acetyl-CoA and malonyl-CoA in the polyketide pathway. These enzymes synthesize exceptionally diverse compounds, which include aflatoxins, lovastatins, and tetracycline (antibiotics) [12,13]. In the NRPs pathway, NRPs are synthesized by using NRPs synthetases. The synthesis of cyclic peptides, like penicillin and cyclosporin, commonly recognized for their antibacterial and immunosuppressive properties, is attributed to these multi-modular enzymes. The terpenoid pathway is an alternative route in which terpenes are produced by the mevalonate or non-mevalonate methods, which involve terpene synthases. Fungal terpenes, such as trichothecenes and fumonisins exhibit antifungal and toxic properties and are crucial for the organism's defense. In alkaloid pathways, fungal alkaloids, like ergot alkaloids, are derived from amino acid precursors and involve a series of decarboxylation and oxidation reactions. These N-containing compounds are known for their pharmacological and toxic effects. In hybrid pathways, certain SMs are produced through hybrid pathways that combine elements of the above pathways, which may lead to the development of complex compounds with unique biological activities. Hybrid pathways indicate the versatility and adaptability of secondary metabolism in fungi [14]. These biosynthetic pathways are usually organized into gene clusters, where the genes encoding enzymes, transporters, and regulators required for SM production are co-located, facilitating coordinated expression. Table 1 summarizes the key pathways involved in the production of SMs in fungi based on the literature.

2.2. Regulatory mechanisms governing metabolite production

Both internal and external signals tightly regulate the production of fungal SMs. Regulatory mechanisms include global regulators, cluster-specific regulators (CSR), epigenetic regulation, and environmental factors, which are described below in brief [9]. Global regulators include transcription factors that control the expression of multiple SMs gene clusters. Examples include the velvet complex (VeA, VelB, LaeA), which regulates developmental processes and secondary metabolism in response to environmental stimuli like light and nutrient availability. CSR are transcription factors that are specific to individual BGC. For example, AflR regulates the aflatoxin biosynthetic gene cluster, controlling the synthesis of aflatoxin in response to specific signals. In epigenetic regulation, chromatin structure also plays a significant role in the regulation of SM production. Histone modification and DNA methylation can either activate or repress gene clusters involved in metabolite synthesis. This allows fungi to fine-tune production based on environmental conditions [19]. Environmental (external) factors, such as temperature, pH, nutrient availability, and interactions with other organisms can influence SM production. Fungi produce antibiotics and toxins to compete with other bacteria during nutritional constraints.

2.3. Recent insights from omics studies

Recent advances in genomes, transcriptomics, proteomics, and metabolomics have illuminated fungal SM biosynthesis and control. These studies have yielded genomic sequencing [20], transcriptomics, metabolomics, and synthetic biology discoveries [21]. Figure 1 shows omics technologies and their importance in fungal SM production.

The biosynthesis and regulation of fungal SMs are highly complex processes that involve multiple pathways and regulatory mechanisms. Recent knowledge from omics studies is expanding our understanding of these processes, which offers new opportunities for discovering novel bioactive compounds and enhancing their production for various applications.

3. Production and regulation of secondary metabolites from fungi

Fungi produce a diverse range of various SMs, which are essential for their existence, ecological interactions, and competitive activities. Several environmental parameters, including nutrition availability, light intensity, and pH, precisely control the synthesis of SM. Moreover, complex genetic pathways and signaling molecules regulate their biosynthesis and make the fungi capable of adjusting to various habitats and stressful environmental conditions. The detailed investigation of these regulatory systems is crucial for effectively using fungal SMs in biotechnology and medicine [22,23].

3.1. Production of fungal secondary metabolites

No doubt, the synthesis of SMs by fungi is a very complex process that is greatly affected by the environment, but they are not required by the fungi directly for their growth. But these SMs produced by fungi become important for interacting with the environment. The SMs (mycotoxins, antibiotics, and other bioactive compounds) are widely used in pharmaceuticals, biomedical, and agricultural fields.

Industrially, fungal SMs could be manufactured by mainly two routes, i.e., submerged-state fermentation (SMF) and solid-state fermentation (SSF) [24,25]. SMF in a liquid medium with a higher volume is referred to as SMF. Upon reaching a fast growth stage, the developing microorganism is transferred to a fermenter tank filled with a growth media whose volume ranges from 30,000 to 200,000 L. SSF is the growth of microorganisms in water-deprived media, utilizing the solid support matrix as a growth medium [26]. The materials that make up the support matrix consist of agro-industrial wastes (sugarcane and cassava bagasse), cereal grains (rice, wheat, barley, and corn), and de-oiled seed cakes (coconut oil cakes, soybean cake, and groundnut oil cake) [27,28]. It has been studied that the developmental process of fungi is linked to the production of SMs; for example, the synthesis of SMs is interlinked with the production of spores by fungi. The SMs associated with the sporulation process are classified into three broad categories (1) sporulation-inducing metabolites (e.g., linoleic acid from *Aspergillus*

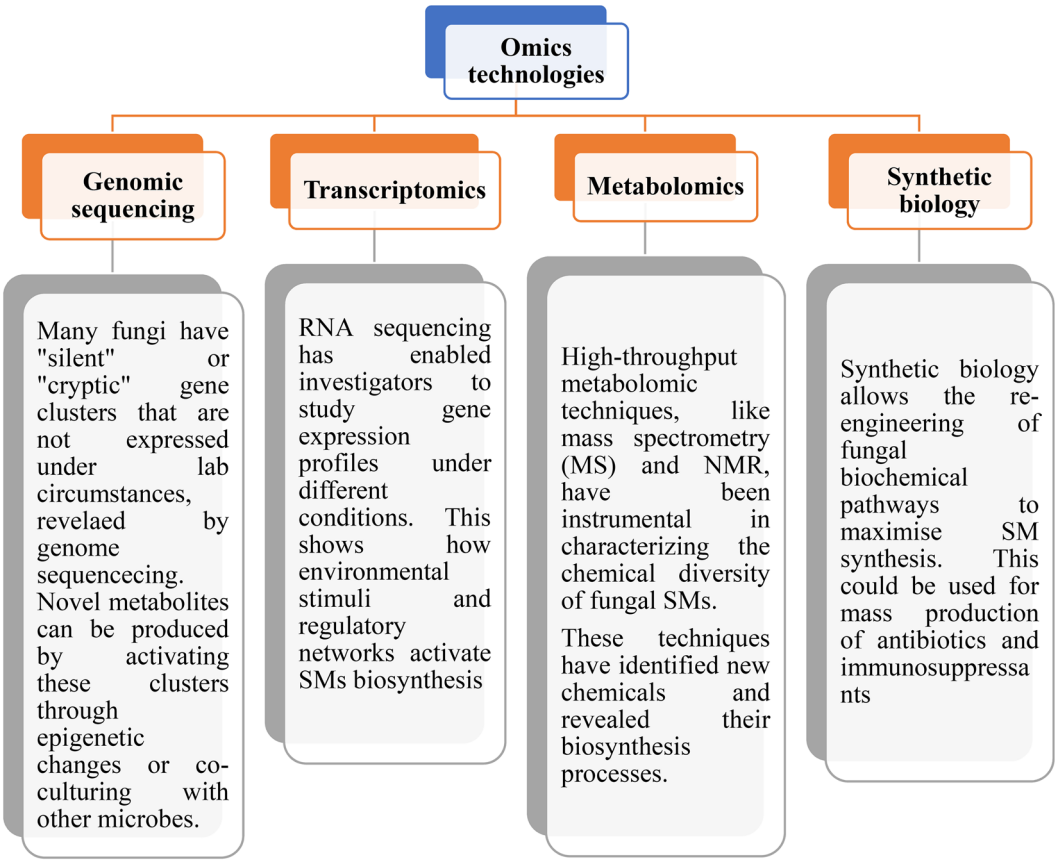

Figure 1. Omics technologies and their importance in fungal SMs production.

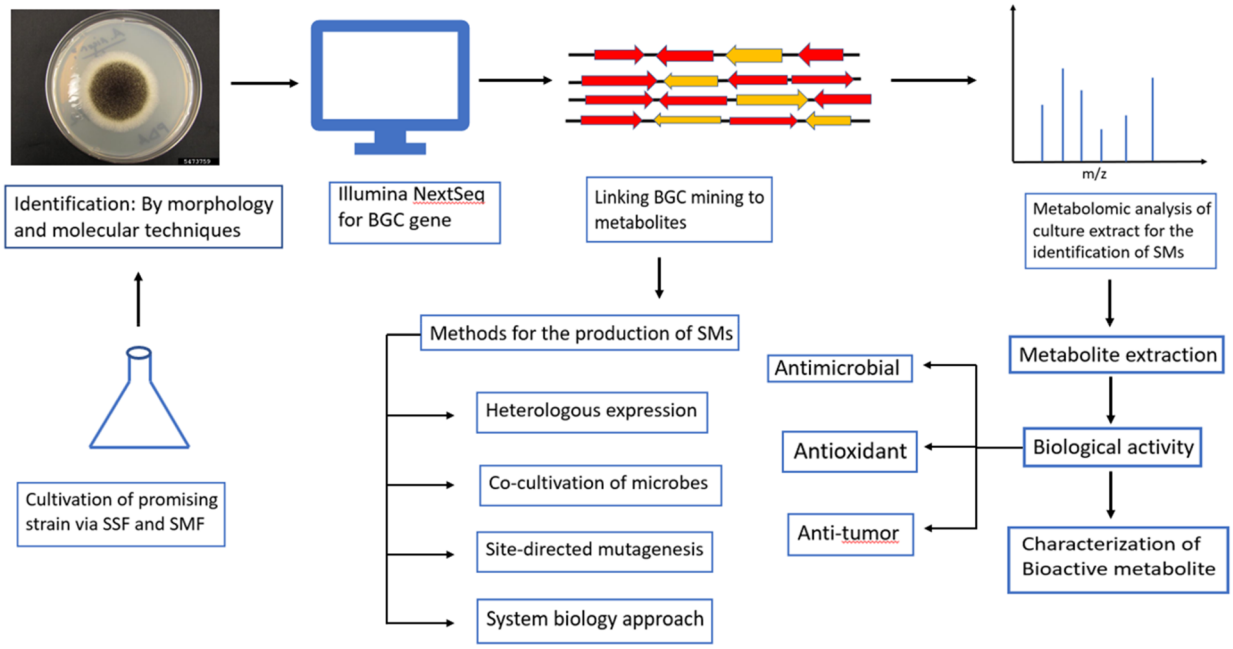

Figure 2. Schematic diagram showing the screening and production of SM from fungi.

nidulans) [29,30], (2) Pigments necessary for the maintenance of spore integrity (e.g., melanin is required for both sexual and asexual spores) [30,31], and (3) Release of toxic metabolites at the time of sporulation to protect them from natural predators (mycotoxins). Figure 2 shows the schematic diagram showing the screening and production of SM from fungi.

3.2. Regulation of production of fungal SMs

The regulation and production of these compounds can be enhanced through genetic and biotechnological approaches like genetic regulation of SMs (LaeA and velvet proteins), heterologous production systems (yeast-based systems), optimization of production conditions (experimental design and chemometrics), and role of fungal endophytes (phenolic compounds) [32].

LaeA and velvet proteins are key regulators of fungal secondary metabolite (SM) biosynthesis. LaeA, with its methyltransferase activity and velvet proteins, which form complexes, influences SM gene expression, particularly in pathogenic fungi, such as *Aspergillus* and *Fusarium*. These proteins are crucial in modulating the production of mycotoxins and other metabolites [33]. Additionally, *Saccharomyces cerevisiae* serves as an efficient host for the heterologous synthesis of fungal biosynthetic gene clusters (BGCs) in yeast-based systems [34]. This approach facilitates the production and study of fungal-derived compounds, such as Colletochlorin SMs [35].

Optimizing the production of fungal SMs can be achieved through systematic experimental designs and chemometrics. Modifying growth conditions, along with advanced techniques like high-performance liquid chromatography-ultraviolet-visible-mass spectroscopy (HPLC-UV-visible-MS), further enhance the yield and quality of these metabolites, making them suitable for various industrial uses [36]. Moreover, fungal endophytes are rich in phenolic SMs, which possess antioxidant, anti-inflammatory, and antibacterial properties. These metabolites, produced *via* the shikimate pathway, play a vital role in plant-microbe interactions. Genetic, biotechnological, and analytical approaches are crucial to unlocking the potential of these compounds for agricultural and medicinal applications [37,38].

4. Diversity of fungal secondary metabolites

The SMs formed by fungi are diverse and include various classes and structures where each one of them has unique environmental and functional properties. The biosynthetic pathway of these fungal SMs belongs to a uniform network of primary metabolism [39]. These fungal SMs belong to mainly four main chemical families, namely terpenoids, polyketides (PK), NRPs, and hybrid non-ribosomal peptide/polyketides (NRP/PKs) [40]. The substrates from primary metabolism are typically utilized to synthesize SMs, with acetyl-CoA serving as the primary precursor to polyketides and terpenoids. These fungal SMs play a crucial role in the interaction of fungi with their environment, including defense mechanisms, communication, and survival strategies. Table 2 shows the detailed diversity of fungal SMs along with biological activity.

Table 2. Different types of fungal secondary metabolites and their biological activity.

Class of metabolite	Examples	Producing fungi	Biological activity	References
Oligopeptides	Cyclosporine A	*Tolypocladium inflatum*	Immunosuppressive	[39,41]
Terpenes	Various	*Tolypocladium, Drechslera, Fusarium*	Antimicrobial, phytotoxic	[41,42]
Polyketides	Various	*Fusarium, Alternaria*	Anticancer, phytotoxic	[42,43]
Alkaloids	Ergot alkaloid	Hypoxylaceae	Antimicrobial, anticancer	[39]
Phenols	Various	*Aspergillus, Chaetomium*	Antioxidant	[44]
Nitrogenous metabolites	Various	*Fusarium, Alternaria*	Phytotoxic	[42]
Saponins	Not specified	Endophytic fungi	Anticancer, anti-inflammatory	[11]
Flavonoids	Various	Endophytic fungi	Antioxidant, anti-inflammatory	[11,44]
Xanthones	Various	Endophytic fungi	Anticancer, antimicrobial	[11]
Quinones	Various	Endophytic fungi	Anticancer, antimicrobial	[11]
Antibiotics	Penicillin	*Penicillium notatum*	Antibacterial	[8,39]
Statins	Lovastatin	*Aspergillus terreus*	Cholesterol-lowering	[39]

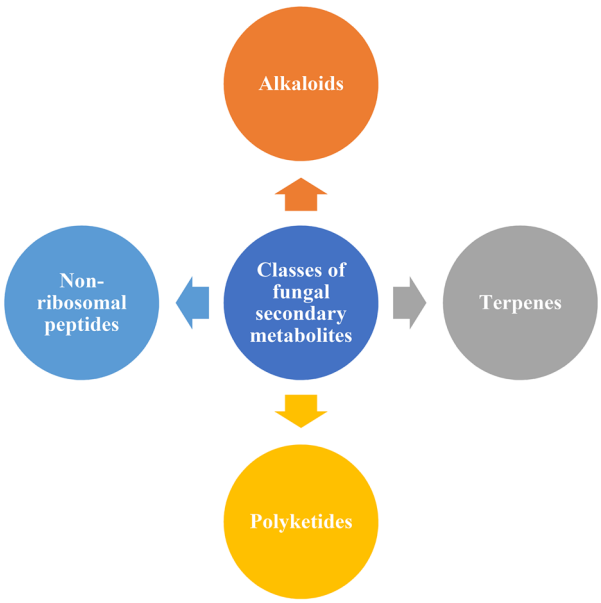

Figure 3. Different classes of SMs of fungi.

4.1. Classes of fungal secondary metabolites

Fungal SMs can be broadly classified into several categories based on their biosynthetic origins, such as alkaloids, terpenes, polyketides, and NRPs [8,45], as shown in Figure 3.

4.1.1. Alkaloids

Alkaloids are nitrogen-containing compounds that are often derived from amino acids and exhibit a wide range of biological activities, including antimicrobial and anticancer properties. Fungal alkaloids, such as ergot alkaloids are well-known for their toxic and medicinal properties. Alkaloids are a major class of fungus SMs with several biological and therapeutic uses. Fungi synthesize these compounds in their secondary metabolism, which is not involved in their growth or reproduction.

Alkaloids are produced in large quantities by marine and endophytic fungi. In particular, the marine fungus is believed to have a lot of alkaloids that can combat inflammation, cancer, and free radicals. Analytical techniques like *in situ* colony assays and LC-MS/MS have been used for the screening and identification of strains with high alkaloid production potential. For instance, *Penicillium mallochii* produces azaphilone alkaloids with notable bioactivities [46].

Alkaloids derived from both EF and non-endophytic fungi structurally belong to indole, quinoline, and diketopiperazine alkaloids, each exhibiting distinct biological properties [47]. Besides this, EF also produces a wide range of alkaloids that have anti-cancer, anti-malarial, and antiviral activities. These compounds enhance the host plant's resilience against plant diseases [11]. Even today, there are a lot of hurdles in the isolation, characterization, and synthesis of fungal alkaloids.

4.1.2. Terpenes

Terpenes are a heterogeneous group of SMs that fungi, plants, and certain bacteria chemically synthesize. These organic compounds consist of isoprene units, like trichothecenes and fumonisins, that exhibit both antibacterial and toxic properties [48]. Terpenes are one of the major fungal organic compounds

which are synthesized *via* intricate biosynthetic methods. These compounds exhibit a diverse array of biological applications, rendering them highly valuable for various applications. Terpenes produced by fungi are synthesized by enzymes called terpene synthases, which consist of different cyclases and enzymes that add functional groups to cyclized structures. Effective control of these enzymes is essential for guiding the synthesis of terpenes, which includes the mevalonate pathway, gene expression, and the availability of cofactors [48].

Recent advances in metabolic engineering and synthetic biology have facilitated the synthesis of terpenes in *S. cerevisiae*. This yeast can be genetically modified to produce a large amount of terpenoids [49]. Terpenes contribute significantly to the regulation of microbial populations, encompassing the growth of both fungi and bacteria. Their effects on the rhizobiome and plant-microbe interactions are terpene-specific, dose-dependent, and can be either stimulatory or inhibitory [50]. The EF-producing terpenes provide defense to host plants from various plant pathogens [51]. Besides this, these terpenes may also have a negative impact on the microbial population, which might be beneficial for the plants and soil.

4.1.3. Polyketides

Polyketides, a heterogeneous group of fungal SMs produced by the PKS enzyme complex, have important biological and pharmacological properties. Some of the common examples of fungal SM as polyketide are aflatoxin (mycotoxins) and lovastatin. Aflatoxin is highly toxic while lovastatin is a pharmaceutical compound used to lower the level of cholesterol [52]. The polyketides are mainly synthesized by *Drechslera*, *Fusarium*, and *Alternaria* spps. Polyketide is a broad class of fungal SMs, where about 61 compounds are phytotoxic in nature, which hinders the growth of the plant. The phytotoxic mechanisms of these fungal polyketides are mainly due to the suppression of germination and root growth, which makes them a potential candidate for the formulation of bioherbicide [42].

Structure-wise, polyketides are very diverse and could vary from a simple linear chain to complex cyclic structures [53]. For instance, *Aspergillus* species produce polyketides which exhibit therapeutic activity and varied chemical structures [52]. Li et al. recently extracted new polyketides having indanone-type and chromone dimer structures from *Penicillium* species [54].

4.1.4. Non-ribosomal peptides (NRPs)

Non-ribosomal peptide synthetases are enzymes that produce NRPs, such as cyclosporin and penicillin, which are significant bioactive molecules of immunosuppressive and antibacterial characteristics, respectively [55]. NRPs are a prominent class of fungal SMs that show various biological activities along with their applications. Fungi are a major source of NRPs, which are commonly used in therapeutics and industries due to their distinctive structural and functional properties. Like polyketides, fungal NRPs are also structurally diverse which could be linear peptides with different ends, cyclic peptides, and depsipeptides. This structural variation in the NRPs is mainly due to their flexible composition, due to which these SMs could be integrated into different amino acids and other substrates into the peptide chain [56]. NRP synthetases are multi-enzyme complexes that function separately from ribosomal machinery to synthesize these peptides. The different domains of this enzyme are responsible for specific steps in peptide synthesis. The selection and activation of the amino acids are carried out by the adenylation (A) domain. The thiolation (T) domain is responsible for the transfer of these substrates to the condensation (C) domain, whereas the peptide bond formation is facilitated by the condensation (C) domain. This modular assembly line mechanism enables the formation of complex peptides without the requirement of ribosomal machinery.

4.2. Structural and functional diversity of fungal SMs

Structurally, the fungal SMs are very diverse and vary from simple molecules to complex macromolecules with complicated ring systems and functional groups [57]. The broader spectrum of the structures translated into functional diversity results in substantial changes in biological activity, even from little changes in chemical structure. Specifically, the polyketides family includes both toxic substances (aflatoxins) and medicinally valuable molecules (statins). Furthermore, terpenes (gibberellins) act as a regulator of plant growth, while others have strong toxic properties. This diversity in fungi is mainly due to their possession of various biosynthetic networks. This enables the fungo to synthesize compounds that are well-suited for a broad spectrum of environmental applications.

4.3. Ecological roles and evolutionary adaptations

Fungal SMs provide diverse environmental functions, which include protection against predators and rivals, symbiotic activities, and communication and signaling. A multitude of SMs function as

toxins to prospective predators, competitors, and pathogens. For instance, mycotoxins can inhibit the growth of competing microorganisms. In some cases, fungi use SMs to develop symbiotic relationships with plants and animals. Besides this, lichens (a symbiotic association between fungi and algae) produce SMs that protect the association from environmental stress. Some of the fungi may produce fungal volatile organic compounds (VOCs) that serve as communication signals within fungal communities or between fungi and other organisms in their environment. From an evolutionary perspective, the ability to produce diverse SMs has likely been a key adaptation for fungi, which enables them to occupy a broad range of ecological niches. The evolutionary pressure to survive in competitive environments has driven the development of these complex biosynthetic pathways. This allows the fungi to modulate their interactions with other organisms and their surroundings.

5. Applications of the fungal secondary metabolites

The SMs of the fungus are widely used in various fields like agriculture, medicine, pharmaceutical, environmental cleanup, and food industries. Their importance is regularly increasing every day. SMs produced by fungi are heterogenous named as antibiotics, anti-tumor, anti-inflammatory, antioxidants, pigments, dyes, polysaccharides, vitamins, glycolipids, polyhydric alcohols, and industrial enzymes (Figure 4). The molecular structures of bioactive compounds or their activities are reported through the usage of recent high-throughput techniques.

5.1. Biotechnological advances in fungal metabolite production

Fungal SMs, renowned for their vast array of bioactive properties, have long been exploited for their industrial, agricultural, and pharmaceutical applications. However, the natural production levels of these metabolites are often insufficient to meet large-scale demands. Recent biotechnological advances, including genetic engineering, fermentation optimization, synthetic biology, and improved bioprocessing techniques, have significantly enhanced the production efficiency and scalability of fungal metabolites. This article explores these cutting-edge developments and their impact on fungal metabolite production.

5.2. Genetic engineering and metabolic pathway manipulation

One of the most transformative advances in fungal metabolite production has been the application of genetic engineering techniques [58]. The manipulation of genes implicated in the biosynthesis of SMs enables researchers to augment or reroute metabolic flow, therefore maximizing the production of targeted chemicals. Primary methodologies encompass gene overexpression, gene knockouts, heterologous expression, as well as CRISPR-Cas9 and gene editing algorithms.

5.2.1. Gene overexpression

The expression of the genes that are responsible for the encoding of the enzyme that limits the rate of biosynthesis pathways could be increased. This may,

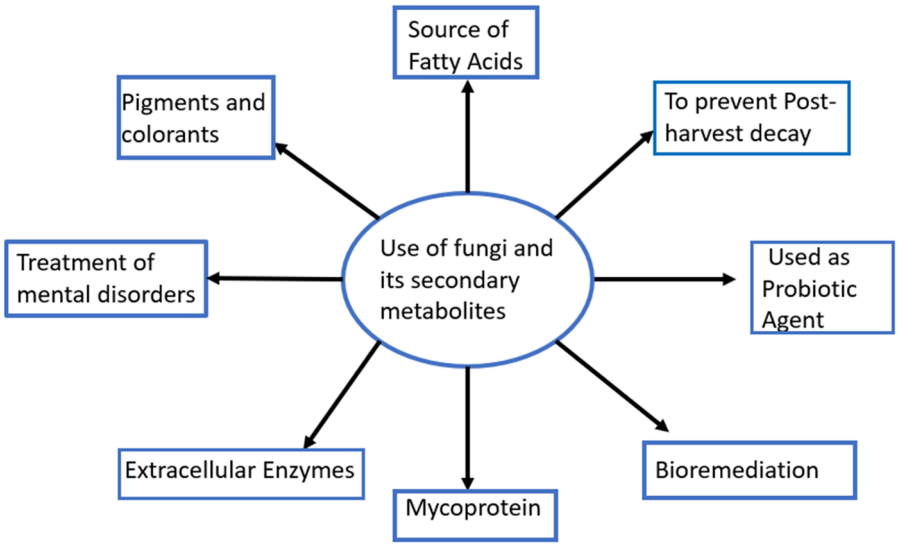

Figure 4. Use of fungi and their SMs.

in turn, increase the yield or output of the metabolite significantly. So, to achieve the increased production of polyketides and NRPs, one has to increase the expression of PKS or NRPS genes, respectively.

5.2.2. Gene knockouts

By applying this technique, the gene expression in fungi can significantly increase the production of SMs by reallocating metabolic resources from competent pathways. This method is especially efficient in increasing the production of targeted bioactive substances (antibiotics and toxins). Under certain conditions, the deactivation of competing pathways can redirect metabolic resources toward the synthesis of the intended molecule. For instance, the elimination of regulatory or competing biosynthetic genes can augment the synthesis of particular antibiotics or toxins. The application of CRISPR-Cas9 in *Penicillium crustosum* enabled the development of an expression platform that increased the efficiency of gene targeting and decreased the SMs background, therefore increasing the synthesis of desired molecules [59].

From the various pieces of literature, it has been found that filamentous fungi have several BGCs that are responsible for the synthesis of SMs. So, to promote the synthesis of specific SMs, one can modulate these BGCs. This includes both pathway-specific and universal regulatory systems, which gene knockout programs can specifically address [9]. Although gene knockouts provide a potential approach to increase SM production, there are still several hurdles to overcome [60,61].

Based on the provided facts, it can be inferred that gene knockouts in fungi are a potent method to increase the production of medicinal mushrooms. Through the deliberate selection of regulatory and biosynthetic pathways, scientists can enhance the production of important chemicals. Nevertheless, the intricate nature of fungal metabolic networks requires meticulous deliberation and exact genetic manipulation to attain the intended results.

5.2.3. Heterologous expression

Heterologous expression of BGCs in hosts with higher genetic tractability is an effective approach to increase hormone synthesis and identify new derivatives. This technique is especially advantageous for the expression of cryptic or silent gene clusters in fungi that provide difficulties in cultivation. Metabolic engineering can greatly enhance production efficiency and expedite the identification of novel chemicals by selectively targeting certain genes within biosynthetic pathways.

An implementation of a highly effective expression platform is necessary. The construction of a *P. crustosum* expression host demonstrates the feasibility of heterologous expression platforms. Utilizing CRISPR-Cas9 technology and genetic dereplication techniques, scientists have improved the efficiency of gene targeting and decreased the presence of SM background, therefore enabling the expression of fungal genes [59]. Yeast has a GEMbLeR system, which enables the enhanced expression of the gene *via* recombinase-mediated rearrangement of promoter/terminator sequences. From the various studies, it is revealed that the production titers of astaxanthin increased by twofold increases by this approach. This suggests its efficacy in enhancing route flow and metabolite yields [62]. The heterologous synthesis of the Colletochlorin family of fungal SMs was mainly carried out by using *S. cerevisiae*. The investigators have connected these metabolites to their biosynthetic genes by using polycistronic routes, which resulted in easing the synthesis and isolation of disease-specific SMs [35]. The precise location of BGCs in the genome, especially within the *Aspergillus* genus, is very important for their expression. BGCs are commonly present in low-synteny and sub-telomeric regions, where histone post-translational modifications enhance gene expression variability. Such information can direct the deliberate choice of host strains for heterologous expression [49].

5.3. Fermentation technologies and optimization

Fermentation technologies play an important role in the mass production of fungus-derived SMs. In the last couple of years, fermentation techniques have emphasized optimizing conditions for maximum yield and productivity through various strategies, such as batch, fed-batch, continuous fermentation, media optimization, and bioreactor design and co-cultivation.

Novel fermentation methods, including fed-batch and continuous fermentation, have been devised to increase the growth of the fungi and metabolite synthesis. Applying the fed-batch fermentation, in which there is an incremental addition of nutrients during cultivation, serves to sustain ideal growth conditions, hence extending the production phase of SMs.

The process of fed-batch fermentation is highly efficient in regulating the provision of nutrients, which results in enhanced synthesis of certain biological compounds. In one of the studies, it was found that when nitrogen utilization was provided in the fed-batch method then there was a significant

increase in the formation of polyhydroxybutyrate (PHB). This indicates the capacity of the fed-batch process to optimize the metabolite yield [63]. When the pretreated sugarcane was fermented by semi-synchronous saccharification in a fed-batch method, there was an increased yield of bioethanol. This demonstrated the efficacy of fed-batch fermentation in sustaining high conversion rates and product yield [64].

Continuous and semi-continuous fermentation techniques provide alternate approaches for the production of biomass. The cultivation of *Trametes versicolor* using semi-continuous fermentation resulted in a significant increase in the production and glucan content. However, the total biomass was lower than that obtained by batch methods [65]. Continuous culture systems, such as those employed for anaerobic gut fungi, enable meticulous regulation of growth rates and metabolic fluxes, resulting in optimized synthesis rates of diverse metabolites in comparison to batch methods [66].

Batch fermentation remains a key technique in biotechnology, with dynamic models offering insights into the metabolic processes of *Saccharomyces* species. These models help optimize fermentation by explaining phase transitions and SM production. While fed-batch fermentation improves growth conditions and metabolite yields, continuous and semi-continuous modes offer benefits like higher productivity and precise metabolic control. The choice of fermentation mode depends on the specific production needs and desired outcomes.

5.3.1. Media optimization

The yield of the metabolite is significantly affected by the composition of fermentation media. The optimal levels of carbon, nitrogen, and trace element sources in the media can significantly increase the synthesis of the particular metabolites. For instance, the composition of glucose and N is crucial in controlling the production of antibiotics and toxins in certain fungi. The C and N sources influence the synthesis of fungal SMs.

Maximizing the efficiency of C sources, like glucose, is crucial in guiding the metabolic flow toward specific products. For example, during the synthesis of 2,3-butanediol, elevated initial glucose concentrations were used to redirect the flow of carbon toward the synthesis of the levo-isomer, therefore improving both the yield and purity.

Various investigations have shown that N sources (tryptone and peptone) have a substantial influence on the synthesis of antibiotics from fungi. Highly increased concentrations of tryptone and peptone were important for optimizing the antibacterial efficacy of *Geotrichum candidum*.

5.3.2. Bioreactor design

Improvements in bioreactor design, including enhanced oxygenation, agitation, and pH regulation, have resulted in more effective manufacturing processes. In large-scale manufacturing, submerged fermentation is the favored approach over SSF because it allows for superior control of environmental parameters.

5.3.3. Co-cultivation

Co-culturing fungi with other microorganisms' mimics natural ecological interactions and boosts SM production. Activating obscure biosynthetic pathways and increasing metabolite production has worked with this method.

These technological developments have significantly increased the efficiency and scalability of metabolite production by fungi, making it more sustainable and profitable.

5.4. Synthetic biology approaches

Synthetic biology offers a powerful toolkit for reprogramming fungal metabolic pathways and designing custom biosynthetic routes for SM production. Using genetic methods and biosynthetic modules, synthetic biology can develop new metabolic pathways or optimize existing ones. This field has advanced pathway reconstruction, modular genetic design, genome editing, and optimization of the framework [67].

5.4.1. Pathway reconstruction

The formation of new biosynthetic pathways in modified fungal strains can be achieved by the assembly of biosynthetic genes derived from various species. Recently, this method has been employed to generate novel categories of metabolites that exhibit heightened bioactivity or enhanced pharmacological characteristics.

5.4.2. Modular genetic design

Novel biology facilitates the modular construction of gene circuits, therefore enabling meticulous regulation of metabolite synthesis. One application of inducible promoters is the precise adjustment of gene expression for important biosynthetic genes, therefore guaranteeing the best circumstances for production.

5.4.3. Genome editing tools

The application of CRISPR-Cas9 technology has completely transformed the capacity to conduct precise genetic alterations in fungi. This software facilitates the accurate modification of BGC, allowing for the activation of inactive pathways and the removal of unwanted byproducts.

5.4.4. Chassis optimization

Contemporary synthetic biology methodologies also prioritize the optimization of fungal strains to function as "chassis" for the synthesis of metabolites. By augmenting the resilience and metabolic efficacy of these strains, scientists can amplify the generation output of valuable secondary compounds.

The integration of synthetic biology with fungal biotechnology is unlocking new possibilities for the production of high-value metabolites, including those that are difficult to extract from natural sources.

5.5. Advances in bioprocessing and scale-up

Efficient bioprocessing and scaling-up of fungal metabolite production is crucial for transitioning laboratory discoveries into industrial-scale operations. Recent advances in bioprocessing technologies have focused on enhancing production efficiency, reducing costs, and maintaining product quality during large-scale manufacturing [36]. Key approaches include downstream processing, process intensification, scalability, and automation and control systems, which are briefly explained.

5.5.1. Downstream processing

The purification and extraction of fungal SMs from intricate fermentation media can provide significant challenges. Recent developments in downstream processing techniques, including HPLC, membrane filtration, and supercritical fluid extraction, have enhanced the potential to obtain pure metabolites with minimum isolating losses [68].

5.5.2. Process intensification

Immobilized fungus, continuous culture systems, and integrated bioprocessing have been employed as strategies to increase the productivity of fungal SMs. The objective of process intensification methods is to minimize the duration of production, decrease energy usage, and optimize the recovery of desired chemicals.

5.5.3. Scalability

Scaling up from lab to industrial fungal metabolite production is difficult. Genetic engineering, bioreactor design, and optimized fermentation conditions have scaled up the production of commercially important metabolites (antibiotics, statins, and immunosuppressants).

5.5.4. Automation and control systems

Automated bioprocess parameter monitoring and control technologies have improved the repeatability and efficiency of the production of SM at large-scale. Automation ensures high-quality production by adjusting pH, temperature, and oxygen levels in real time.

By applying a collective approach to genetic engineering, advanced fermentation technologies, synthetic biology, and improved bioprocessing techniques, it is possible to gain huge production of SMs from fungi. These biotechnological innovations are not only increasing the yield of valuable compounds but also enabling the discovery of novel metabolites with potential applications in pharmaceuticals, agriculture, and biotechnology.

6. Applications of fungal secondary metabolites in agriculture

Fungal SMs have gained huge attention in agriculture for their potent biological activities and environmental benefits. The SMs address key agricultural challenges like pest control, plant growth, and disease management [58]. By decreasing the need for synthetic pesticides, fungal SMs promote sustainable farming, improve crop productivity, and serve as biopesticides. Through biotechnological advancements, these SMs enhance crop development and disease resistance, which offers great potential for sustainable food security.

6.1. Fungal metabolites as biopesticides

Synthetic pesticides are harmful to the environment, yet fungal metabolites are natural. These chemicals are effective against insects, nematodes, and diseases, making them useful in integrated pest management. Biopesticides made from fungal metabolites are insecticidal, antibacterial, and nematocidal.

6.1.1. Insecticidal activity

Insecticides from fungal metabolites damage insect nervous systems or metabolism. Various fungal strains produce these compounds as a natural pesticide alternative, addressing insect resistance and environmental impact. Fungal SMs like oxalic acid, beauvericins, and dextrixins kill insects by penetrating their exoskeletons. Entomopathogenic Fungi (EF) produce pest-fighting chemicals. Additionally, cyclic

and linear peptide toxins derived from primary metabolites have been reported to have specific insecticidal actions. Metabolites modify insect cell membranes, cause fluid loss, impact molting and metamorphosis, and kill insects. These metabolites can be used in agriculture to kill insects, boost plant resistance, and integrate into sustainable pest management. Beauvericin, a toxic cyclic peptide, paralyzes and kills insect pests in *Beauveria bassiana*. *Metarhizium anisopliae* metabolites kill locusts, aphids, and other crop pests. Some fungal SMs have been shown to kill autumn armyworms. *Clodosporium cladosporioides* and *Verticillium lecanii* extracts demonstrated effective toxicity with LC50 values of 229 and 341 ppm, respectively, suggesting their potential as natural pesticides.

These metabolites affect pest physiology by altering enzyme activities and damaging larval tissues, such as the cuticle and midgut lining, which are crucial for pest survival [69].

Arunthirumeni et al. isolated nearly 20 different bioactive compounds by using ethyl acetate as a solvent from the *Penicillium* sp. and tested for insecticidal activity on *Spodoptera litura* and *Culex quinquefasciatus* larvae. The larvicidal activity of *Penicillium* sp. showed significant larval mortality after 48 h of exposure with LC_{50}: 72.205 mg/mL: LC_{90}: 282.783 mg/mL and LC_{50}: 94.701 mg/mL: LC_{90}:475.049 mg/mL, respectively. The high antifeedant activity was observed in 300 µg/mL at 48 h of crude extract exposure. The present study concludes that *Penicillium* sp., SMs are effective in control of *S. litura* and *C. quinquefasciatus* larvae [70].

6.1.2. Antimicrobial properties

Fungal metabolites can also serve as natural fungicides, inhibiting the growth of pathogenic fungi that cause plant diseases. These metabolites, produced by fungi, exhibit diverse bioactivities, such as fungicidal, bactericidal, insecticidal, and herbicidal properties, aiding in the control of plant pathogens. They offer a natural alternative to chemically synthesized biocontrol agents, addressing the challenges in plant disease management. Aflatoxins, produced by *Aspergillus* species, have shown potential in controlling fungal diseases in plants when applied in low concentrations.

Afifa et al. emphasized the potential of fungal-derived bioproducts for sustainable pharmaceutical applications, highlighting their value-added properties. The investigator highlighted the importance of bioprospecting in discovering new fungal-derived bioproducts that could be utilized in the pharmaceutical industry for various applications [71].

Conrado et al. reviewed that fungi species like *Pestalotiopsis neglecta* and *Pestalotiopsis versicolor* isolated from the Japanese Yew tree produced paclitaxel with significantly higher yields compared to previous reports. This highlights their potential as sources of this compound for drug discovery. The investigators further highlighted that entomopathogenic fungi strains have the potential to be a promising source of antimicrobial compounds, as shown by the SMs obtained from 342 strains in Korea that completely suppressed the growth of specific bacteria [72].

Overall, fungal SMs show promise in revolutionizing agricultural practices for sustainable plant disease control.

6.1.3. Bioherbicides

From the various pieces of literature, it has been proven that fungal SMs could be used as bioherbicides for weed management. The SMs derived from fungi offer promising applications in agriculture as bioherbicides, by providing an eco-friendly alternative to chemically synthesized herbicides. SMs having features of bioherbicides are mainly produced by *Drechslera*, *Fusarium*, and *Alternaria*. These SMs also have phytotoxic properties that can effectively target and manage weed populations. Such fungal-based bioherbicides are gaining popularity due to their specificity, reduced environmental impact, and lower risk of resistance development in comparison to traditional chemical herbicides. Advanced technologies like nanomaterials are being explored for formulating these fungal SM-derived products. Fungal bioherbicides demonstrate a high degree of specificity by selectively targeting certain weed species or growth stages, hence decreasing unintended damage to non-target plants and creatures. Fungal bioherbicides have a far faster degradation rate in the environment compared to synthetic herbicides, therefore diminishing their persistence and accumulation in soil and water bodies. This feature contributes to the conservation of biodiversity and the sustainability of the environment [73]. Kuldeep and Sahu emphasized that investigators and farmers are progressively using fungal bioherbicides as a viable substitute for synthetic herbicides in the effective control of weeds. Fungal bioherbicides provide desirable characteristics including precise targeting, minimal ecological footprint, and decreased likelihood of resistance formation in comparison to synthetic herbicides, rendering them a highly attractive choice for sustainable farming methods [73].

Seychelles et al. examined 183 phytotoxic fungal SMs and categorized them into five categories: polyketides, terpenoids, nitrogenous metabolites, phenols and phenolic acids, and miscellaneous chemicals.

Further research found that these compounds prevent weed germination, root, and vegetative growth, and tissue and organ changes in plants [42].

Torres et al. found that herbicide use has caused weed resistance, prompting the search for more environment-friendly approaches like microbial bioherbicides. Further research suggested using cell-free metabolites to improve the efficacy and commercial viability of microbial bioherbicides, which emphasized the need to optimize manufacturing methods and use alternate C and N sources for sustainability [74].

Evidente et al. highlighted the application of fungal phytotoxins as potential bioherbicides to control weeds and parasite plants in agriculture. Investigator also highlighted the commercial availability of bioherbicides based on microbial toxic metabolites, providing insights into their application in the field and mode of action for more sustainable weed control practices in agriculture.

6.1.4. Nematocidal action

Fungal SM plays a crucial role in agriculture by demonstrating nematocidal action. These compounds are derived from microorganisms and have shown effectiveness in reducing populations of phytopathogenic nematodes. Some of the fungal metabolites target parasitic nematodes, which are major agricultural pests and offer an environmentally friendly alternative to chemical nematicides. Such fungal-derived SMs exhibit diverse mechanisms to control plant-parasitic nematodes (PPNs). The nematocidal mode of action of these fungal SMs acts through various mechanisms, which include direct toxicity to nematodes, disruption of nematode development, and interference with nematode-host interactions. These compounds can inhibit nematode egg hatching and immature survival, by decreasing the population of the nematode effectively [75,76].

Sharma et al. cultivated *Purpureocillium lilacinum* on karanja deoiled cake medium to produce SMs which exhibited nematocidal action against *Meloidogyne incognita*, inhibiting egg mass hatching and affecting second-stage juveniles. Some of the major compounds identified in the filtrate by GCMS, such as 2-ethyl butyric acid, phenyl ethyl alcohol, benzoic acid, benzene acetic acid, and 3,5-Di-t-butylphenol, have potential applications in agriculture for the sustainable management of root-knot nematodes. The most potent fraction (fraction 14–15) obtained from column chromatography of the ethyl acetate extract of *P. lilacinum* exhibited 94.6% egg mass hatching inhibition on the 5th day and a maximum nematicidal activity of 62% against second-stage juveniles after 48 h at 5000 mg/L [77].

Dai et al. isolated seven metabolites and identified from *Harposporium anguilla*, including a new polyketone compound 5-hydroxy-3-(hydroxymethyl)-6-methyl-2H-pyran-2-one (1) and six known metabolites. The fungal SM terpendole C from *H. anguilla* exhibits weak nematicidal activity against the root-knot nematode *M. incognita*. Additionally, a new polyketone compound from the same fungus, 5-hydroxy-3-(hydroxymethyl)-6-methyl-2*H*-pyran-2-one, shows an attractive effect toward nematodes. These findings suggest that fungal SMs like terpendole C and polyketones have potential applications in agriculture for their nematocidal actions, indicating a promising avenue for the development of biocontrol agents against nematode pests. Compound 1 exhibited an attractive effect toward the nematode *Panagrellus redivivus*, indicating that some SMs from *Harposporium anguillulae* are involved in the pathogenicity process of infecting nematodes [78].

Farhat et al. isolated endophytic fungi *Aspergillus terreus*, *Cephalosporium* sp., *Chaetomium* sp., *Curvularia lunata*, *Curvularia hawaiiensis*, *Macrophomina phaseolina*, *Fusarium solani*, *Talaromyces assiutensis*, and *Talaromyces trachyspermus* from healthy plants and evaluated for nematicidal activity against *Meloidogyne javanica*. *In vitro*, culture filtrates of these fungi showed strong nematicidal activity by killing the young nematode to varying degrees, whereas *F. solani* caused 100% mortality after 48 h. This indicates that fungal SMs, such as those produced by endophytic fungi like *F. solani*, have the potential to act as nematicides in agriculture. The application of the fungal derived products offers a unique opportunity to discover novel therapeutic agents to combat various pathogens and agricultural pests [79]. Besides this compound like chaetoglobosin and hirsutellone, produced by *Chaetomium* and *Hirsutella* species, respectively, have demonstrated significant nematicidal activity.

A team led by Lei investigated the nematocidal activity of 2-furoic acid generated by *Dactylellina haptotyla* during nematode entrapment. This compound showed high nematicidal action against *M. incognita*, with an LD50 value of 55.05 µg/mL after 48 h. The results of the pot experiment revealed a considerable decrease in the number of galls on tomato roots in the experimental group treated with 2-furoic acid. This demonstrates the potential of 2-furoic acid as a biocontrol agent against plant root-knot nematodes [80].

Arif et al. showed that fungal SMs can be formed in the rhizosphere and contribute to PPN biocontrol. Fungi are capable of effectively controlling the nematode populations by producing SMs, which stimulate the interaction of fungi with plant roots,

providing a long-term solution for nematode control in agriculture.

The biopesticides derived from fungi are a potential approach for sustainable pest management as they target specific pests while having a low influence on non-target organisms and the environment.

6.2. Role of fungal secondary metabolites in plant growth promotion and disease resistance

In addition to pest control, fungal metabolites promote plant development and increase disease resistance. Several beneficial fungi produce SMs that promote plant development and help plants survive environmental stresses and diseases.

6.2.1. Growth promotion by modulation of plant-fungal interactions

Bioactive compounds produced by fungi can stimulate the growth of roots, improve the absorption of nutrients, and boost the general growth of plants. Gibberellins, fungal terpenes, are known as plant growth regulators that promote the elongation of stems, germination of seeds, and development of fruits. Moreover, indole-3-acetic acid (IAA), which is synthesized by a specific soil fungus, promotes the extension and propagation of roots, therefore enhancing the uptake of nutrients. The diverse functions of fungal SMs in stimulating plant growth encompass the regulation of plant-fungal relationships, augmentation of plant defense mechanisms, and emulation of plant hormones [81].

6.2.2. Induced systemic resistance (ISR)

Fungal metabolites induce systemic resistance (ISR), which helps plants resist illnesses after fungal colonization. Plants and fungi interact extensively due to metabolic and structural modifications. Antifungal and antibacterial properties of SMs prevent infections and control plant stress [82,83]. For instance, the production of volatile organic compounds (VOCs) by fungi like *Trichoderma* and *Piriformospora* stimulates plant defense mechanisms, hence increasing resistance to fungal and bacterial infections.

ISR involves biochemical and structural defense, phytohormones, gene expression, and metabolic changes. Biochemical defenses include phytoalexins, disease-associated proteins, and SMs, whereas structural defenses involve cell wall changes and protective coverings [84]. Principal phytohormones, including jasmonic acid and ethylene, play a vital role in ISR by serving as signaling molecules that initiate defense reactions. These hormones facilitate the production of SMs and enzymes that enhance the immunity of the plant [85,86]. Colonization by fungi can induce alterations in the expression of plant genes, therefore augmenting the ability to fight future infections. For instance, the colonization of soybean by a hypovirulent fungus resulted in the up-regulation of genes related to both PAMP-triggered immunity and effector-triggered immunity, as well as the biosynthesis of jasmonic acid [87].

It is concluded that ISR could play a major role in sustained plant protection and enhancement of crop growth and yield. ISR presents a highly promising approach for achieving sustainable agriculture by reducing dependency on chemical pesticides [84]. The utilization of mycovirus-infected hypovirulent fungal strains has demonstrated promise in increasing plant immunity, which resulted in increased plant growth and resistance to disease [87].

6.2.3. Mimicking plant hormones

Fungal-produced SMs can mimic plant hormones like auxins, gibberellins, and abscisic acid, aiding plants in stress tolerance and growth regulation [3]. SM, which mimics the plant hormones, also helps in stress tolerance and growth modulation of the plants. Basit et al. suggested that fungal SMs like isoprenoids, carotenoids, and flavonoids play a vital role in improving plant stress tolerance. These compounds facilitate the adaptation of plants to abiotic stimuli, such as drought, temperature variations, and salinity by regulating stress-related physiological processes [88]. Moreover, several fungal SMs imitate plant hormones, therefore stimulating root and shoot growth and improving disease resistance by activating systemic defenses in plants [3].

6.2.4. Mycorrhizal fungi

Metabolites produced by arbuscular mycorrhizal fungus (AMF) are crucial to plant growth and resistance. These fungi establish symbiotic relationships with plant roots, exchanging nutrients and improving plant health. AMF-generated microbial metabolites help plants transport nutrients between the fungus and plant, resist drought, salinity, and disease, and synthesize beneficial chemicals. SMs derived from the AMF also protect the plants from root diseases [89].

According to Weisany et al., AMF colonization significantly boosts the production of bioactive compounds in plants, which are essential for medicinal uses and human health. This enhancement is attributed to improved nutritional conditions and protection provided by AMF, which results in increased SM production in medicinal plants [90].

In some cases, it has been found that AMF has increased nutrient uptake and stress tolerance. AMF improves nutrient acquisition, particularly P, which is vital for plant growth. Through modulation of antioxidant defense systems and hormonal regulation, this symbiotic connection improves plant tolerance to abiotic challenges, such as drought, salinity, and heavy metal toxicity [91]. It has been observed that the introduction of AMF has enhanced the nutritional composition of maize seeds by raising the concentrations of organic acids, amino acids, and fatty acids. Furthermore, AMF enriches antioxidant characteristics, as seen by elevated levels of polyphenols, flavonoids, and ascorbic acid [92].

Certain studies have shown that AMF provides resistance to foliar fungal infections by modifying the structure of the root system and regulating reactive oxygen species. This, in turn, decreases the need for chemical fungicides and encourages an environmentally benign method of plant protection [93]. Furthermore, AMF also enhances soil health by stimulating soil structure and nutrient cycling, therefore promoting resilient ecosystems and sustainable agricultural practices [91]. Furthermore, it has also been observed that AMF can help in the mitigation of heavy metals and arsenic pollutants. In a particular study, it was shown that AMF effectively mitigated the buildup of heavy metals, like antimony and arsenic, within plants. Consequently, this led to a reduction in their concentration within plant tissues and an enhancement in plant health. These reductions are essential for preserving plant productivity and ensuring safety in polluted environments [94].

6.3. Enhancing crop productivity through biotechnological means

Biotechnological innovations using fungi's SMs might improve agricultural yield. Fungal metabolites can improve plant health and productivity due to their biological activities. These natural substances can reduce the use of chemicals in agriculture, making crop management easier and more eco-friendly. Genetic engineering, fermentation, and synthetic biology are optimizing fungal SM production and integrating it into agriculture.

6.3.1. Genetic engineering of beneficial fungi

Genetic engineering of beneficial fungi is promising for increasing the synthesis of compounds that promote plant growth and prevent pests. Through the manipulation of genetic pathways in fungi like *Trichoderma* and *Beauveria*, scientists have successfully enhanced their biocontrol capacities and boosted the synthesis of advantageous biological SMs. This approach improves the efficacy of this fungus in managing biological pests and promotes sustainable agriculture.

The biological control of plant diseases by *Trichoderma* species is widely acknowledged. They synthesize NRPs, polyketides, and other SMs that are essential to their biocontrol. Genetic engineering can increase metabolite production, improving biological pesticide efficacy [95]. *Beauveria bassiana* is used as a biological pesticide since it parasitizes many arthropods. Gene modifications can increase siderophores, which are essential for iron metabolism virulence and stress resistance. Its ability to circumvent host protective mechanisms may enhance pest management [96,97].

Fungal species like *Trichoderma* and *Beauveria* produce SMs that affect plant growth and stress tolerance. These pathways can be altered through metabolic engineering to increase isoprenoids and flavonoids, which help plants tolerate abiotic stresses [58,98,99]. Recombinant DNA was used for genetic engineering. This method has helped genetically modify fungi to secrete more proteins and SMs. This has resulted in fungal strains with superior biocontrol and plant growth promotion [58]. Genetic engineering of beneficial fungi has huge potential, but it must be considered environment-friendly and not harm the environment.

6.3.2. Fermentation and formulation technologies

Advances in fermentation technologies have significantly enhanced the large-scale production of fungal metabolites for agricultural applications. These technologies leverage various fermentation methods, genetic engineering, and precision fermentation to optimize the production processes, making them more efficient and sustainable. Formulation technologies are also being developed to create stable, long-lasting products that retain their bioactivity when applied to crops. Encapsulation techniques, for instance, can improve the shelf life and efficacy of fungal biopesticides.

To date, different fermentation techniques have been applied and optimized; for instance, submerged fermentation, SSF, and co-culture are employed to produce fungal metabolites. These methods are particularly effective in utilizing agricultural waste as a substrate, which is abundant and noncompetitive with food resources. Process optimization, including the use of genetic engineering, can enhance enzyme production, meeting industrial demands without significant additional investment [100].

The integration of metabolic and protein engineering, systems biology, and synthetic biology has led to the discovery of novel metabolic pathways. These advancements facilitate the production of primary and SMs, like organic acids and hydrolytic enzymes, which are important for agricultural applications.

Precision fermentation, which includes techniques like next-generation sequencing and CRISPR-Cas9, optimizes microbial strains and metabolic pathways, improving product yields and bioprocess scale-up [101].

The task of scaling up fermentation processes continues to be difficult. However, investigation on *Aspergillus fumigatus* has demonstrated that increasing agitation speed can increase metabolite production on a larger scale [102]. Scaling up fungal biopolymer production, which has considerable agricultural benefits, requires careful culture medium and selection of conditions for production [103].

Although these advancements provide encouraging possibilities for sustainable farming approaches, difficulties like the energy-intensive preprocessing of substrates and the complexity of expanding their use persist. Ongoing research and technical advancement are very important to overcome these challenges and fully harness the potential of fungal SMs in agriculture.

6.3.3. Synthetic biology approaches

Synthetic biology has improved fungal metabolic pathway modifications, promoting the synthesis of novel or improved fungal metabolites. By applying this approach, one can manipulate and optimize fungus metabolic processes to produce valuable compounds for various sectors. Optimizing synthetic biology gene clusters (BGC) for metabolite synthesis may improve bioactive compound yield or produce agriculturally beneficial derivatives. These transformed metabolites may be more effective, have more diverse activity, or be more stable in the field [102].

The refactoring of the BGC is feasible. An innovative synthetic biology technology has been created to convert BGCs into monocistronic transcriptional units. It enables the cloning and integration of biosynthetic genes into *Streptomyces* chromosomes, therefore enabling the synthesis of aromatic polyketides, such as landomycin and mithramycin [104]. Employing heterologous expression platforms, such as the one developed in *P. crustosum*, amplifies the production of fungal structural proteins. This platform utilizes CRISPR-Cas9 technology to enhance the efficiency of gene targeting and minimize the presence of metabolite background, therefore facilitating the effective expression of PKS genes [104].

Optimization of microbial cell factories for the effective synthesis of SMs is also feasible. Novel synthetic biology techniques have been used to enhance the efficiency of microbial cell factories, namely *S. cerevisiae*, in generating valuable metabolites. Through the manipulation of noncoding sequences and the optimization of metabolic flow, scientists have substantially enhanced pathway activity and product outputs [105]. Plasmid copy number modification in *S. cerevisiae* increases fungal polyketide synthesis. By achieving equilibrium in PKS subunit expression, novel compounds could be isolated and product yields raised tenfold [106]. Synthetic biology has produced food additives, colors, and antibiotics by genetically modifying fungus strains. This methodology fills gaps in fungal biology knowledge and improves fungal chassis efficiency in industrial applications [107].

Despite synthetic biology's success in reorganizing fungal metabolic pathways, challenges persist. Understanding fungal biology and developing more effective genetic techniques is required. If these issues are resolved, fungi will be better biofactories for important SMs.

6.3.4. Microbial consortia

Microbial consortia, which use several beneficial fungi and bacteria, may increase agricultural output, especially in challenging environments. These consortia boost crop yields through biopesticide actions, plant growth stimulation, and nutrient availability [108].

Microbial consortia increase crop stress tolerance. Microbial consortia help plants tolerate abiotic stressors including drought, according to numerous research [109]. AMF, yeasts, and rhizobacteria improved the rate of photosynthesis and levels of nutrients in strawberry plants, improving drought tolerance [110]. Furthermore, microbial consortia reduce phosphate stress. NEER-PHOS, a consortium of phosphate-solubilizing bacteria, increased growth and reduced chemical fertilizer use in Vigna radiata and Cicer arietinum [111].

Microbial consortiums promote nutrient absorption and growth [112]. Microbial consortia improve nutrient solubilization and absorption. For instance, the NEER-PHOS mixture made phosphate soluble, fixed nitrogen, and solubilized potassium, boosting plant growth [111]. A consortium of *Trichoderma afroharzianum*, *Azotobacter chroococcum*, and a fungal SM increased sweet basil yield and photosynthetic activity. This suggests that such formulations

boost crop yields [113]. In such microbial consortium, fungal SMs, such as those produced by *Trichoderma* species, play a significant role in modulating plant defense mechanisms and promoting growth. These metabolites can enhance stress tolerance and improve plant health by acting as biocontrol agents and inducing systemic resistance [113,114].

6.4. Antagonistic yeast: To prevent postharvest decay and decomposition of fruit

Fruits are an essential part of the human diet, but fungal pathogens seriously harm them when they are being transported and stored. The postharvest spoilage of fruit by fungi includes rot, nutrient, and water loss that causes economic loss. During their growth, fungi produce mycotoxins which can enter the food chain and can be harmful to human health. A highly necrotrophic fungal pathogen, *Botrytis cinerea,* grows aggressively on the tissues of more than 200 plant species, including vegetables and fruits. The primary pathogenic agent, *B. cinerea*, causes grey mold and affects a variety of crops at the postharvest stage, including tomatoes, strawberries, and raspberries. *Penicillium* rots caused by *Penicillium digitatum*, *Penicillium expansum*, and *Penicillium italicum* are other examples of a devastating fungal disease that affects crops after harvest. *Penicillium expansum* is a necrotrophic fungus that causes blue mold, causes blue mold which has a major impact on orchard fruits, primarily apples, while *P. digitatum* and *P. italicum*, cause postharvest green mold, also known as green rot in citrus fruits. *Penicillium expansum* is defined as a wound pathogen that penetrates or invades the fruit tissue by using brushes. Apart from these negative effects, *P. expansum* produces mycotoxin named patulin, a teratogenic, carcinogenic, immunotoxin compound in apples that can harm human health [115]. For several years, chemical fungicides have been used to control postharvest spoilage, but their massive usage leads to environmental pollution and the emergence of highly resistant fungal pathogens. Recently, one of the groups has also isolated drug-resistant *Candida auris* (one of the five pathogens proclaimed as the most dangerous by the U.S. Centers for Disease Control and Prevention in 2019) from stored apples, which can prove to be dangerous transmission reservoirs [116]. However, its role in postharvest decay is yet to be seen. Thus, there is an urgent need to design a novel and safe approach to control postharvest infestation by pathogenic fungi.

Over the past few decades, great work has been done to explore the biocontrol capability of microorganisms against postharvest decay. Antagonistic yeasts have been used as commercial products because of their excellent performance. Antagonistic yeasts are called biocontrol yeasts that inhibit the growth, development, and reproduction of phytopathogens. To avoid postharvest rot and degradation of fruits, antagonistic yeasts have emerged as a promising biocontrol method, providing an environmentally benign alternative to synthetic fungicides. The yeasts in question counteract spoiling by suppressing harmful fungi, therefore mitigating food loss and waste [117]. An optimal antagonistic yeast for combating postharvest decay should possess genetic stability, be capable of growth under unfavorable conditions, exhibit a wide spectrum of activity against phytopathogenic fungus, have low nutrient requirements, lack the production of any harmful metabolites, and be easily stored and dispensed [118,119].

The antagonistic yeasts exert their effects through several mechanisms, such as the production of toxins and fungal volatile organic compounds (FVOCs), rivalry with other yeast species for resources and space, and mycoparasitism [120]. Several instances exist in which the antagonist yeast has effectively impeded the proliferation of the infection. For instance, *Hanseniaspora uvarum*, *Papiliotrema terrestris*, and *Rhodosporidium glutinis* have shown effectiveness against pathogens like *Botrytis cinerea* and *Alternaria alternata* by suppressing spore germination and mycelial growth. *Haemophilus uvarum*, specifically, synthesizes antifungal volatile organic compounds (VOCs) that impede the growth of pathogens [121].

In some cases, the antagonist yeast forms a biofilm on the fruit and prevents spoilage. For instance, *Rhodotorula glutinis* yeast has demonstrated effective biocontrol against green mold decay in oranges caused by *P. digitatum*. The *R. glutinis* rapidly colonizes fruit wounds, forming a stable population that inhibits pathogen growth. The biocontrol efficacy is dose-dependent, with higher concentrations of *R. glutinis* leading to better control of the pathogen. The yeast's ability to maintain high population levels even at low temperatures (4 °C) further underscores its potential for use in cold storage conditions [122]. *Pyrenochaeta terrestris* produces high levels of extracellular lytic enzymes, which contribute to pathogen suppression [123]. Besides this, FVOCs can alter pathogen cell morphology and disrupt cellular processes, leading to cell death. These compounds are

considered safer and less toxic alternatives to chemical pesticides [124].

So far, many antagonistic yeasts have been identified and also developed as commercial products, such as *Candida* spp., *Cryptococcus* spp., *Metschnikowia* spp., *Pichia* spp., *Rhodotorula* spp., *Candida oleophila*, *Candida sake*, *Metschnikowia fruticola* and yeast-like fungus *Aureobasidium pullulans* [120], *S. cerevisiae* and *Cryptococcus albidus*. They all have been used against postharvest pathogens *Rhizopus stolonifer*, *Penicillium* spp., *Collectotrichum* spp., *Botrytis cinerea*, *Monilinia fruticola*, *Aspergillus niger*, and *A. alternata* [125].

There are several advantages of antagonistic yeasts for agricultural applications. Antagonistic yeasts are nontoxic, low-residue, and environmentally friendly, addressing consumer concerns about chemical fungicides [126]. By reducing postharvest losses, antagonistic yeasts contribute to sustainable agriculture and food security, aligning with the U.N. Sustainable Development [127]. Table 3 outlines key fungal SMs and their applications in enhancing agricultural productivity and plant health, either by acting as biopesticides, biocontrol agents, or improving plant stress tolerance.

From the above information, it was found that fungal metabolites hold immense potential in agriculture, providing natural solutions for pest control, plant growth promotion, and crop productivity enhancement. Through ongoing biotechnological advancements, the agricultural applications of these compounds continue to expand, which offers promising results for more sustainable and resilient farming systems.

7. Applications of fungal secondary metabolites in medicine and pharmaceuticals

Fungi are a rich source of bioactive SMs, many of which have been instrumental in transforming medicine and pharmaceuticals. Fungal metabolites have led to the discovery of several life-saving drugs, including antibiotics, anticancer agents, immunosuppressants, and more. The diverse chemical structures and unique biological activities of these compounds make them invaluable for drug development, addressing global health challenges, such as antibiotic resistance, cancer, and autoimmune diseases [140]. Here the significant contributions of fungal metabolites to modern medicine, focusing on

Table 3. Applications of various fungal secondary metabolites in agriculture.

Fungal SMs	Source fungus	Applications	References
2-Furoic acid	*Dactylellina haptotyla*	Nematocidal activity; reduces root galls on tomatoes	[80]
Aflatoxins	*Aspergillus* spp.	Fungicidal activity; controls plant disease	[71]
Avenacin	*Fusarium* spp.	Natural defense compound	[128]
Beauvericin	*Beauveria bassiana*	Biopesticide, insecticidal activity; paralyzes and kills insect pests	[69,129]
Bioactive compounds (ethyl acetate extract)	*Penicillium* sp.	Insecticidal activity on *Spodoptera litura* and *Culex quinquefasciatus*	[70]
Chaetoglobosin, hirsutellone	*Chaetomium* sp., *Hirsutella* sp.	Nematocidal activity	[79]
Cyclosporin A	*Tolypocladium inflatum*	Plant stress tolerance	[130]
Dothistromin	*Dothistroma pini*	Biocontrol of plant pathogens	[131]
Ergosterol	*Saccharomyces cerevisiae*	Induces plant defense responses	[132]
Fumonisins	*Fusarium moniliforme*	Biocontrol agent	[133]
Gibberellins	*Gibberella fujikuroi*	Plant growth regulators, promote stem elongation, seed germination, and fruit development	[81,134]
Gliotoxin	*Aspergillus fumigatus*	Biocontrol of nematodes	[135]
IAA	Soil fungi	Enhances root growth and nutrient uptake	[81]
Iturin A	*Bacillus subtilis* (produced by fungus interaction)	Antimicrobial activity, antifungal activity; inhibits *Fusarium*, *Botrytis*	[71]
Jasmonic acid, ethylene	Various fungi	Induces systemic resistance, enhances plant immune responses	[85,86]
Destruxins	*Metarhizium anisopliae*	Insecticidal activity; effective against locusts, aphids	[69]
Harzianic acid	*Trichoderma* species	Induce plant growth	[95]
Oxalic acid, beauvericins, dextrixins	Entomopathogenic fungi	Insecticidal activity; damages the insect nervous system, metabolism	[69]
Paclitaxel	*Pestalotiopsis neglecta, P. versicolor*	Antimicrobial activity; drug discovery	[72]
Penicillic acid	*Penicillium cyclopium*	Antibacterial agent	[136]
Phytoalexins	Various fungi	Biochemical defense in ISR, stimulating plant immunity	[84]
Polyphenols, flavonoids, ascorbic acid	AMF	Improves antioxidant properties and plant resilience	[92]
Siderophores	*Beauveria bassiana*	Improves iron metabolism, virulence, and stress resistance for biological pest control	[137]
Sirodesmin PL	*Leptosphaeria maculans*	Herbicide/phytotoxin	[138]
Tenuazonic acid	*Alternaria alternata*	Fungicide/phytotoxic properties	[136]
Terpendole C, polyketones	*Harposporium anguilla*	Nematocidal activity	[78]
Trichodermin	*Trichoderma viride*	Antifungal agent	[139]
Volatile organic compounds (VOCs)	*Trichoderma, Piriformospora*	Stimulates plant defense mechanisms; increases resistance to fungal and bacterial infections	[3,83]

antibiotics, anticancer compounds, and the role of fungi in novel drug discovery.

7.1. Antibiotics and antimicrobials from fungal metabolites

Perhaps the most celebrated contribution of fungal metabolites to medicine is the discovery of antibiotics. Fungal-derived antibiotics have revolutionized the treatment of bacterial infections, saving millions of lives worldwide.

Microorganisms are considered an ample source of bioactive compounds having a variety of structural characteristics; the pharmaceutical sector has expanded the scope of antibiotic screening programs over time. Development in the field of recombinant DNA technology and biotechnology helps us to manipulate fungi genomes at their full potential to synthesize SMs. The most important class of antibiotics is defined as beta-lactams, which mainly include penicillin, cephalosporins, and carbapenems. Each of these compounds works by inhibiting the synthesis of a bacterial cell wall layer named as peptidoglycan layer. PcRFX1 global regulatory factor, which is present in the penicillin-producing bacterium *P. chrysogenum*, regulates the expression of three genes, pcbAB, pcbC, and pence, which are involved in the biosynthesis of beta-lactams [141].

One of the first antifungal medications, griseofulvin, was derived from mold named *Penicillium griseofulvum* (Figure 5) and is generally used for the treatment of fungal infections. The drug acts by binding to tubulin and interfering with mitosis and microtubule function [142]. The effectiveness of the drug is limited to dermatophytes only, such as *Trichophyton* species, *Microsporum*, and *Epidermophyton floccosum*. Apart from this, a study was conducted on an alkaliphilic fungus, named *Sodiomyces alkalinus* for the exploration of natural active compounds against human pathogenic and opportunistic fungal strains. Kuvarina et al. extracted novel hydrophobin Sa-HFB1 from *S. alkalinus* and checked its antifungal activity against clinical and non-clinical isolates. The MIC of Sa-HFB1 against fungal pathogens ranged from 1 to 8 μg/mL; the highest antifungal activity (MIC 1 μg/mL) was shown for clinical isolate *Cryptococcus neoformans* [143].

Fungal alkaloids have shown significant promise in pharmaceutical applications. For example, verticillins, a group of epipolythiodioxopiperazine alkaloids, exhibit potent cytotoxicity against cancer cell lines and are being explored for their chemotherapeutic potential. These compounds probably function as specific inhibitors of histone methyltransferases, therefore affecting apoptosis and immune recognition [144].

The diverse range of activity demonstrated by fungal alkaloids, encompassing antibacterial, antifungal, and immunosuppressive properties, highlights their promise as therapeutic agents. Their wide-ranging uses in medicine, particularly in Ayurvedic practices, emphasize the need for additional investigation and application [39].

In addition to their function in stress management, fungal alkaloids serve as defense mechanisms, enabling the fungi to cope with abiotic stressors. This ability to produce a variety of metabolites, including alkaloids, under stress conditions makes

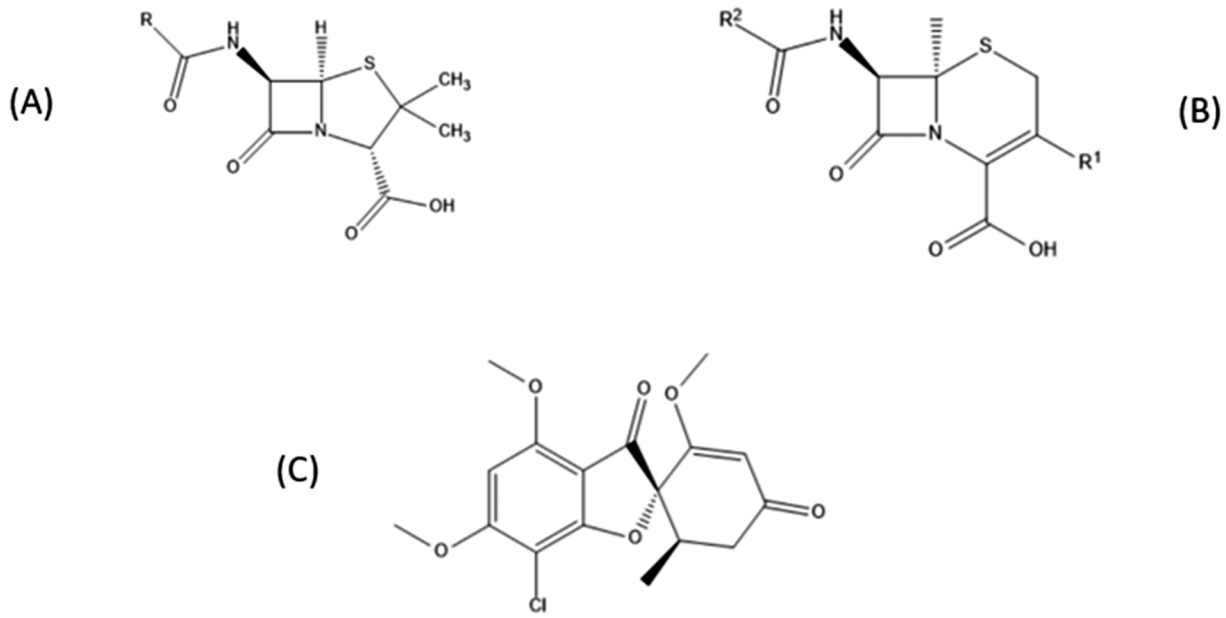

Figure 5. Chemical structures of representative fungal SMs having antimicrobial activity. (A) Penicillin; (B) Cephalosporin; (C) Griseofulvin.

fungi an excellent model for studying stress responses and the ecological roles of SMs [39].

7.1.1. Terpenes: Biological activities and applications

The terpenes demonstrate a wide range of biological actions, encompassing antioxidative, anti-inflammatory, antiviral, and antitumor features. The aforementioned activities render them highly attractive contenders for pharmaceutical applications, including the development of anticancer medications and antibiotics. As a subset of terpenoids, diterpenes are known for their wide range of biological activities and applications in the fields of medicine, agriculture, and personal care [145].

7.1.2. Polyketides: Antimicrobial and anticancer activities

An abundant source of polyketides with substantial antibacterial and anticancer properties is found in endophytic fungi. The inhibitory effects of these compounds on pathogenic microorganisms and cancer cell lines render them highly valuable in the pursuit of drug discovery [146].

7.1.3. Applications of fungal NRPs

Pharmacologically, fungal NRPs have been formulated into medications that have substantial therapeutic advantages. Examples of compounds produced from fungal NRPs and used in clinical conditions include cyclosporin A, an immunosuppressant, and caspofungin, an antifungal drug [147,148].

Fungal NRPs are a heterogeneous collection of bioactive substances that have considerable therapeutic utility owing to their antibacterial, immunosuppressive, and cytostatic properties. Deriving from a variety of fungal sources, including marine and terrestrial fungi, these peptides have played a crucial role in the development of therapies for infections, cancer, and inflammatory disorders. Notable antibiotics derived from fungi include penicillin, cephalosporin, griseofulvin, and echinocandins [149].

7.1.3.1. Penicillin. Its great efficacy against several bacterial illnesses, such as pneumonia, syphilis, and meningitis, stems from its capacity to suppress bacterial cell wall formation. Penicillin and its derivatives, such as amoxicillin, continue to be essential in contemporary medicine despite the increasing prevalence of antibiotic resistance.

Fungal NRPs have been pivotal in antibiotic development, with penicillin and cephalosporin being classic examples derived from *Penicillium* species. These antibiotics have revolutionized the treatment of bacterial infections by targeting bacterial cell wall synthesis [148]. Marine-derived fungal peptides exhibit significant cytotoxic activity against various human cancer cell lines. For instance, peptides from *Aspergillus* and *Penicillium* species have demonstrated potent anticancer properties, making them promising candidates for cancer therapy [150].

7.1.3.2. Cephalosporins. Like penicillin, cephalosporins are β-lactam antibiotics produced by the fungus *Acremonium* (previously known as *Cephalosporium*). These antibiotics are effective against both Gram-positive and Gram-negative bacteria, and their broad-spectrum activity has made them a cornerstone in treating bacterial infections that are resistant to penicillin.

7.1.3.3. Griseofulvin. Derived from the fungus *P. griseofulvum*, griseofulvin is an antifungal antibiotic used to treat dermatophytic infections like ringworm and athlete's foot. Its mode of action involves inhibiting fungal mitosis, making it an important treatment for fungal skin infections. Griseofulvin is primarily used to treat skin infections, such as jock itch, athlete's foot, and ringworm, as well as fungal infections of the scalp, toenails, and fingernails [151].

Beyond its antifungal properties, griseofulvin has shown potential in other areas, such as anti-inflammatory, cardiovascular, antitumor, and antiviral activities [152].

7.1.3.4. Echinocandins. Echinocandins are another class of antifungal agents produced by fungi, such as *Glarea lozoyensis*, which represent a newer class of antifungal drugs. They inhibit the synthesis of β-glucan, an essential component of the fungal cell wall, making them highly effective against invasive fungal infections, particularly *Candida* and *Aspergillus* species [153].

The identification of these fungal antibiotics established the basis for contemporary antimicrobial treatment and remains a crucial field of endeavor, especially in combating antibiotic-resistant diseases.

7.1.4. Anticancerous compounds

Furthermore, fungal metabolites play a significant role in the advancement of anticancer and immunomodulatory chemotherapy. These chemicals demonstrate several modes of action, such as triggering apoptosis in cancer cells, suppression of cell division, and regulation of the immune system. Fungal organisms synthesize a diverse range of secondary metabolites, including amino acids, alkaloids, anthraquinones, and terpenes, that have notable anticancer effects [8,45].

The fungus *Penicillium* and *Aspergillus* are renowned for their ability to produce bioactive chemicals that have promising medicinal uses [8].

The induction of apoptosis in cancer cells by fungal metabolites is a vital mechanism underlying their anticancer action. The efficacy of these compounds in combating medication resistance has been especially observed in pancreatic cancer. The metabolites exert their effects *via* diverse biosynthetic routes, selectively targeting molecular processes exclusive to cancer cells, hence inducing cell cycle arrest and apoptosis [154].

7.1.4.1. Paclitaxel (Taxol). Paclitaxel, initially obtained from the bark of the Pacific yew tree, was subsequently discovered to be synthesized by endophytic fungus, namely *Taxomyces andreae*. Paclitaxel is a potent chemotherapeutic drug that stabilizes microtubules and inhibits the proliferation of malignant cells. It is widely used in the treatment of ovarian, breast, and lung cancers [155,156].

Polyketides isolated from *Penicillium oxalicum* have demonstrated potent inhibitory effects on pancreatic tumor growth, with specific compounds like oxalihexane A showing remarkable cytotoxicity against cancer cell lines.

Behera et al. highlighted the identification of fungal metabolites as potential anticancer agents, offering higher potency, lower toxicity, and increased efficacy compared to existing chemotherapy drugs [157].

7.1.4.2. Sterigmatocystin and other mycotoxins. Sterigmatocystin and other mycotoxins have also shown anticancer activity but they have toxic effects. Sterigmatocystin, a precursor of aflatoxins, has been studied for its potential to induce apoptosis in cancer cells. However, the therapeutic use of such compounds requires careful control due to their toxicity [158].

7.1.5. Mycotherapy: Potential of fungal bioactive compounds for the treatment of mental disorders

There are numerous mental health disorders, including addiction disorders, mood disorders, impulse control issues, eating disorders, obsessive-compulsive disorder (OCD), anxiety disorders, psychotic disorders, post-traumatic stress disorder (PTSD), and personality disorders [159,160]. Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5) 2013 states that "A mental disorder is a syndrome defined by a clinically significant disturbance in an individual's cognition, emotion regulation or behavior that reflects malfunction in the biological, psychological, biological or developmental processes that underlies mental functioning in an individual." According to a WHO report, the prevalence of mental illness is increasing day by day across the world, with 20% of children and adolescents diagnosed with the disorder and suicide being the primary cause of death [161]. The foundation for the treatment of mental health disorders is combinatorial drug therapy that includes mood stabilizers, anti-psychoactive drugs, and psychotherapeutic treatments. In many cases, patients suffering from mental illness frequently do not respond and are termed as "treatment resistant" [162]. The drugs prescribed for the treatment of mental illness exhibit side effects to patients, such as increased suicidal thoughts, declining physical health, and difficulties in the workplace [163,164]. To provide a good therapeutic drug in the market, scientists have brought up novel alternative options to treat mental illness. Fungal biologics have gathered attention for their potential to treat mental health disorders, using psilocybin, magic mushrooms, ergine from rye ergot fungus *Claviceps purpurea*, ayahuasca, and lysergic acid diethylamide (LSD). LSD is defined as a synthetic derivative derived from a precursor called D-lysergic acid. Bioactive compounds isolated from medicinal mushrooms are considered a new source of psychoactive drugs. Additionally, there is a growing interest in supporting medicinal mushroom companies for the development of novel therapeutic drugs by implementing the quadruple helix approach (academia-industry-policy-society). For this, the focus of attention will be based on bioactive compounds having low molecular weight, which can easily cross the blood-brain barrier (BBB) and should have similar structural features to available neurotransmitters [161].

Psychedelics or serotonergic hallucinogens are natural compounds that show antagonism to 5-HT receptors. They produce hallucinogenic responses in brain regions by regulating emotions, perception of pain, and self-awareness [165]. Psychedelics are a subclass of substances called psychoplastogen that are involved in the regulation of brain function. Psychoplastogens can treat PTSD, mood and anxiety disorders [166]. Mushrooms containing these psychoplastogens are used to treat mental illness. Psilocybin, an important component of the hallucinogenic prodrug psilocin, is obtained from a *Psilocybe* mushroom called a magic mushroom. Psilocybin binds to serotonin transporter protein and dopamine receptors with good affinity. Both psilocybin and its metabolite psilocin can pass through BBB, but psilocin is more potent [167]. A study conducted by Gill et al. found that the administration of 10 and 25 mg of psilocybin can effectively alleviate symptoms of anxiety and major depressive disorder in a mentally diseased patient, without any discernible adverse effects [168].

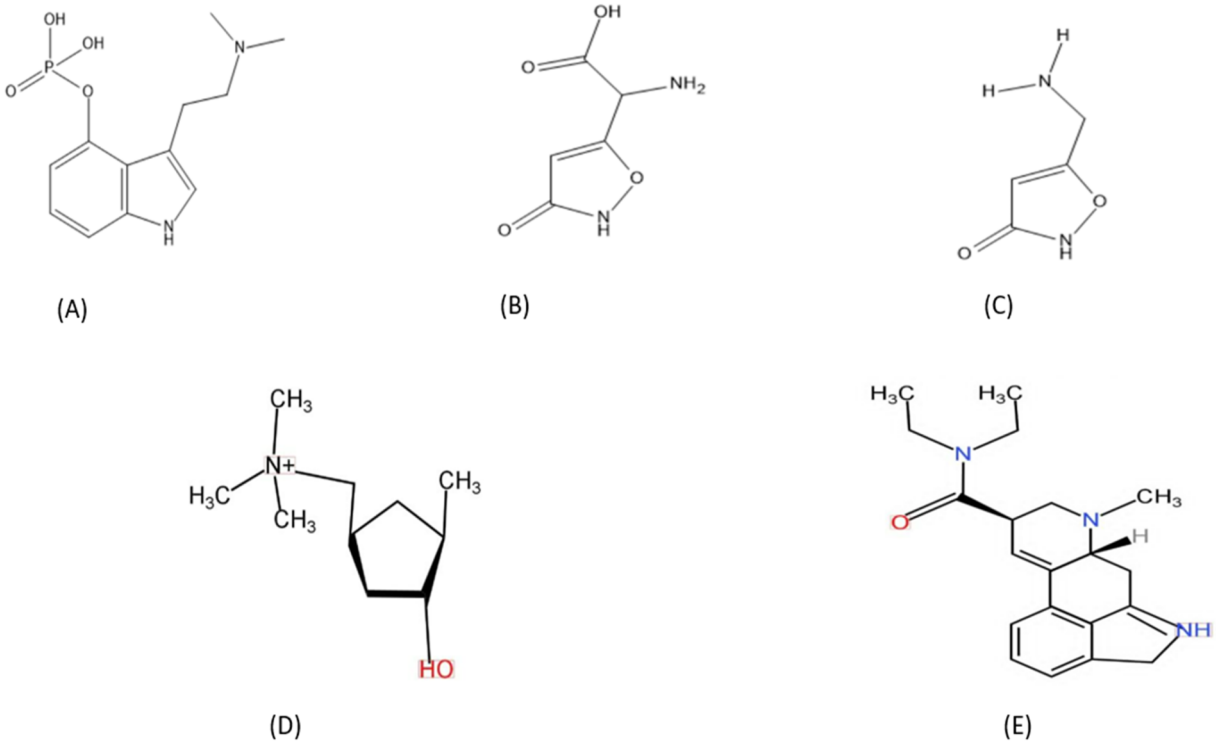

Figure 6. Chemical structure of (A) Psilocybin; (B) Ibotenic acid; (C) Muscimol; (D) Muscarine; (E) Lysergic acid diethylamide (LSD).

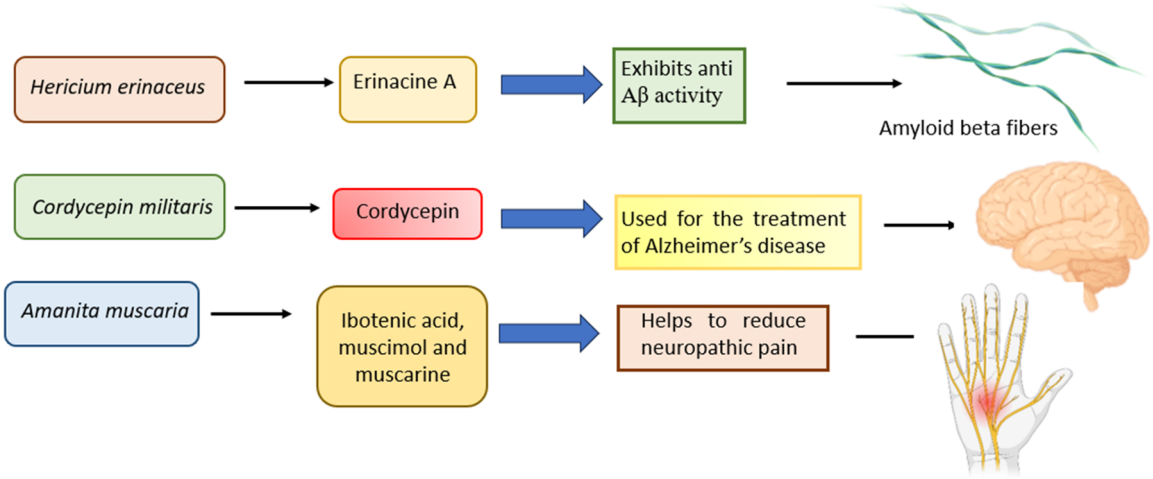

Figure 7. Schematic representation of major mushrooms and their metabolites in the treatment of brain-related disorders.

The fungal species *C. purpurea* is employed in the treatment of Parkinson's disease due to its production of an ergot alkaloid that exerts a notable impact on specific areas of the brain. LSD is a partially synthetic derivative of lysergic acid obtained from *C. purpurea*. The psychedelic drug LSD exerts its effects on perception, emotion, and cognitive functions *via* interacting with serotonin receptors. *Amanita muscaria* produces biologically active compounds, including psychoactive ibotenic acid, alkaloids, muscimol, and muscarine (Figure 6).

Ibotenic acid and muscimol share similar structural features with gamma-aminobutyric acid (GABA), which primarily acts by binding to glutamate receptors in the Central Nervous System (CNS) and can cross the BBB. Muscimol is used in combinatorial therapy with endomorphin-1 to reduce the symptoms of neuropathic pain brought on by spinal cord injuries.

Cordyceps militaris produces an active component called cordycepin; it is defined as 3-deoxyadenosine, a purine analog that exhibits multiple roles in neurodegenerative diseases, including neuroprotective, immunomodulatory, and anti-inflammatory activities [169,170]. Both *Armillaria mellea* (AM) and *C. militaris* (CM) are defined as medicinal mushrooms, and their extracts have been used in the treatment of mood disorders, including anxiety and depression.

They work by inhibiting pro-inflammatory cytokines and stimulating the release of anti-inflammatory mediators by regulating the release of neurotransmitters [171–173].

The medicinal benefit of *Hericium erinaceus* is known for its anti-cancer, anti-inflammatory, antimicrobial, and anti-oxidative activities, but apart from this, neurotropic compounds present in the mushroom are generally used for the treatment of neuropsychiatric disorders. Erinacines and hericenones are the biologically occurring neurotrophic compounds that are found in the fruiting body and mycelium of *H. erinaceus*. These drugs are used to treat Parkinson's and Alzheimer's disease because they influence nerve growth factor (NGF), cross the BBB, and prevent amyloid beta (Aβ) aggregation (Figure 7).

NGF is required for the maintenance of nervous tissue and the functioning of neurons. However, patients suffering from MDD have very reduced levels of NGF, and it is also unable to cross BBB. The neurotrophic compounds that were extracted from *H. erinaceus* promote nerve myelination throughout the CNS and increase the production of nerve growth factor (NGF) in the brain. Enhancement in the levels of NGF and myelination of neurons by mushroom extract helps to reduce MDD, mood, and anxiety disorders.

7.1.6. Immunomodulatory compounds

Fungal SMs have emerged as promising immunomodulatory compounds, offering potential therapeutic benefits in managing immune-related disorders and infections. These metabolites, derived from various fungal species, exhibit diverse chemical structures and biological activities, making them valuable candidates for drug discovery and development. The identification of the cyclic peptide cyclosporine A from *T. inflatum* marked the start of a new chapter in medical history [174]. Cyclosporine (Figure 8) functions by binding with the T-cell receptor, increasing the level of calcium and stimulating the calcineurin [175]. Calcineurin is known as protein phosphatase, which dephosphorylates the transcription factor NF-AT, also called as nuclear factor of activated T-cells. This process leads to activation of T-cell activation by stimulating the expression of genes that encode cytokines and interleukin-2. Cyclosporin works by inhibiting the dephosphorylation of NF-AT by binding to cyclophilin, thus resulting in reduced function of effector T-cells [176].

Cyclosporine is regarded as the first immunosuppressive drug as it regulates the function of T-cells without showing any toxicity. It prevents the rejection of bone marrow, kidney, liver [177], and heart by acting as an immunosuppressant drug.

Research on the endophytic fungus *Aspergillus* sp. has led to the discovery of new immunosuppressive SMs. Compounds, such as pseurotin and diphenolic derivatives have demonstrated inhibitory effects on T-cell proliferation, indicating their potential to modulate immune responses [178]. *Aspergillus fumigatus* produces gliotoxin, a fungal metabolite with potent anticancer properties which induce apoptosis in cancer cells by generating reactive oxygen species (ROS) and disrupting mitochondrial function. In addition to its anticancer potential, gliotoxin exhibits immunosuppressive activity, making it a candidate for autoimmune disease treatments. The dual role of fungal metabolites in targeting cancer cells and modulating the immune system makes them promising candidates for developing novel therapies in oncology and immunology.

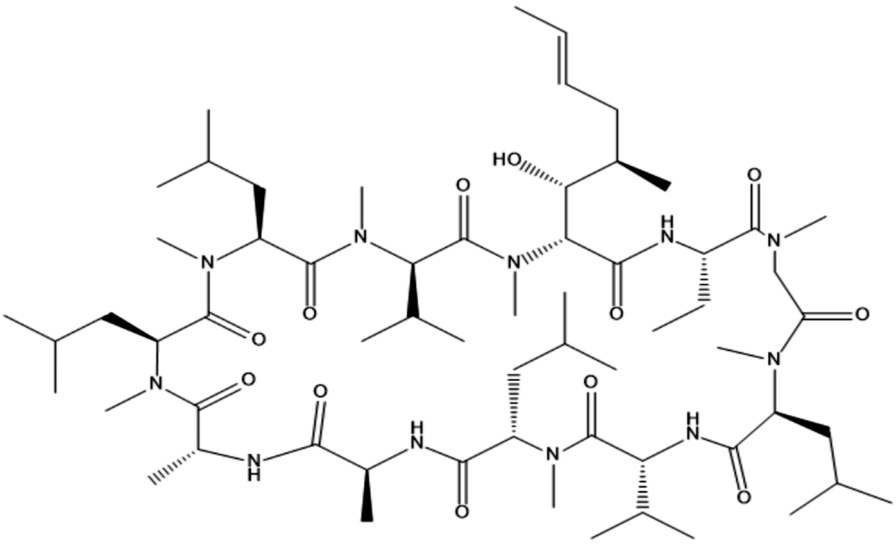

Figure 8. Chemical structure of cyclosporin A.

Table 4. List of fungal secondary metabolites having an anti-immunomodulatory effect.

Fungi	Metabolite type	Immunomodulatory activity	References
Bipolaris maydis	Ophiobolin-type sesterterpenoids	Enhanced phagocytosis, cytokine production, antioxidant activity	[180]
Daldinia sp. TJ403-LS1	Acetylenic phenol derivatives (1, 4, and 7), one benzofuran derivative (8), one new naphthol derivative	Influence the production of cytokines, activation of immune cells, antioxidant properties, modulation of gut microbiota	[179]
Aspergillus sp.	Diphenolic derivatives	Inhibit proliferation of murine T cells	[178]
Aspergillus sp.	Pseurotin	Inhibit proliferation of murine T cells	[178]
Aspergillus sp.	Butenolide derivative	Immunosuppressive effects	[178]
Aspergillus sp.	Benzopyran	Immunosuppressive effects	[178]
Aspergillus sp.	Isochromane lactone	Immunosuppressive effects	[178]
General fungal sources	Various SMs	Immunomodulators and immunosuppressants	[72,185]
Fungal endophytes	Anti-HIV compounds	Potential immunomodulatory and antiviral activities	[186]

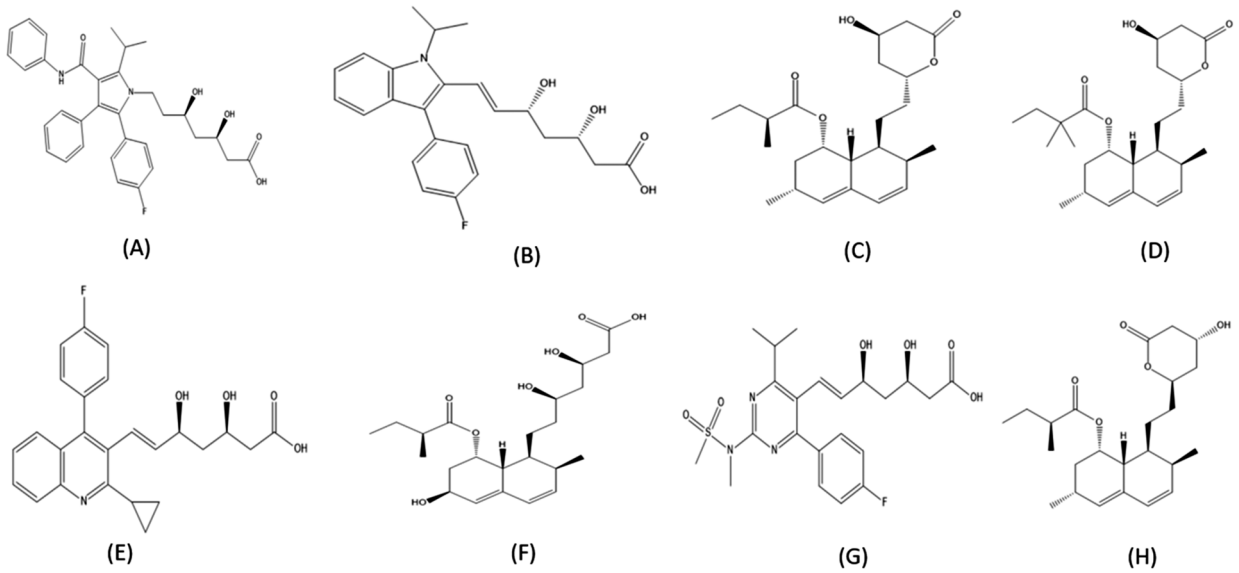

Figure 9. Chemical structure of synthetic analog of statins. (A) Atorvastatin; (B) Fluvastatin; (C) Lovastatin; (D) Simvastatin; (E) Pitvastatin; (F) Pravastatin; (G) Rosuvastatin; (H) Monacolin K.

Lin et al. isolated and identified five new acetylenic phenol derivatives (1, 4, and 7), one benzofuran derivative (8), one new naphthol derivative (9), and two known analogs (5 and 6) from an endophytic fungus *Daldinia* sp. TJ403-LS1, which was isolated from the medicinally valuable plant *Anoectochilus roxburghii*. Their capacity to suppress the immune response rendered the metabolites highly immunosuppressive. Furthermore, the separated metabolites likewise showed inhibitory effects on Butyrylcholinesterase (BChE). BChE inhibitors are relevant in the treatment of neurodegenerative diseases. The dual activity of these metabolites (from *Daldinia* sp. TJ403-LS1), both as immunosuppressants and BChE inhibitors, opens up possibilities for their use in developing new therapeutic agents [179].

Duan et al. isolated and identified new SMs (ophiobolin-type sesterterpenoids) with immunosuppressive activity from the phytopathogenic fungus *Bipolaris maydis*. These newly discovered metabolites have the potential to be further studied for their immunosuppressive properties, which could have implications for the development of new therapeutic agents [180].

The investigations carried out by Seo and Choi, and Raut demonstrated that mushrooms contain various bioactive compounds (polysaccharides, proteins, and triterpenes), which have antiviral activities against a range of viruses (herpes simplex virus, influenza virus, and HIV). These compounds can interfere with viral entry, replication, and protein synthesis, offering effects comparable to conventional antiviral drugs [181,182]. Specific fungal defensins, like micasin, have shown potential in targeting viral components, such as the SARS-CoV-2 spike protein, highlighting the role of fungal compounds in developing novel antiviral agents [183,184]. The antiviral potential of mushrooms is further supported by studies on medicinal mushrooms like *Inonotus obliquus*, which have shown broad-spectrum antiviral activity against influenza viruses. Table 4 shows the list of fungal SMs having an anti-immunomodulatory effect.

7.1.7. Cholesterol assimilation by fungi

Statins represent the most potent class of cholesterol-lowering drugs isolated from *A. terreus* and *Monascus ruber* [187]. The increasing demand for these drugs led to the synthesis of synthetic

analogs of statins called atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, selektine, rosuvastatin, simvastatin (Figure 9). The filamentous fungus *Monascus purpureus* produces pigments, lipids, and monacolins. This fungus is highly known for fermenting white rice to make angkak, also called beni koji, and deep red rice. Traditional Chinese medicines use *M. purpureus* fermented rice to improve blood circulation and heart health. With the advancement of scientific tools, researchers discovered that *M. purpureus* produces monacolin as SMs that can lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which is defined as the rate-limiting step in the biosynthesis of cholesterol. The highly effective bioactive molecule Monacolin K, which is patented as lovastatin, is currently widely employed for the treatment of hypercholesterolemia conditions [188].

7.1.8. Antileukemic agents

Certain fungi produce SMs having antileukemic properties. Monaspin B, a newly developed chemical obtained from the simultaneous culture of *M. purpureus* and *Aspergillus oryzae*, has demonstrated considerable promise as an antileukemic antibiotic. Analysis of the literature reveals that Monaspin B is a naturally occurring compound obtained from *M. purpureus* and another fungus. It has been extensively utilized for different therapeutic applications, such as its anti-inflammatory and neuroprotective properties [189].

By co-cultivating *M. purpureus* and *A. oryzae*, Meng et al. discovered Monaspin B, resulting in the synthesis of two new cyclohexyl-furans, Monaspins A and B. The production process was fine-tuned to achieve a yield of 0.8 mg/L of Monaspin B.

High-resolution ESI-MS and NMR techniques were used to determine the structure of Monaspin B, therefore verifying its completely new chemical structure. Further, the investigator demonstrated potent antiproliferative activity against the leukemic HL-60 cell line, with an IC50 of 160 nM, indicating its strong potential as an antileukemic agent. Performing the *in vivo* studies using a mouse leukemia model demonstrated that Monaspin B effectively reduced white blood cell, lymphocyte, and neutrophil counts, further supporting its therapeutic potential [190].

Monaspin B's have shown promising results for leukemia therapy, and other compounds like Birinapant have shown efficacy in different cancer models, such as melanoma, by targeting apoptosis pathways, suggesting broader applicability of natural and synthetic compounds in cancer therapy [190].

7.1.9. Anti-inflammatory

There are several fungal SMs that have anti-inflammatory properties. To date, several fungal metabolites have been used as anti-inflammatory drugs.

Wu et al. isolated various compounds (four azaphilones, three benzenoids, one benzofuranone, one 5′,6′-dihydrospiro[isochromane-1,2′-pyran]-4′(3′*H*)-one derivative, two steroids, and six tetralones) from *M. purpureus* BCRC 38110. The structures of these compounds were characterized using advanced techniques, such as 1D and 2D NMR, UV, IR, and HRESIMS analyses, which helped in understanding the biological activities of the compound. Further investigators assessed the isolated compound for the anti-inflammatory effect and found that monapurpureusone and monascuspirolide B inhibited the release of inflammatory cytokines IL-8 and TNF-α in human chondrosarcoma cells when induced by interleukin (IL)-1β. This suggested their potential applications in treating inflammatory conditions, such as osteoarthritis [189].

7.1.10. Neuroprotective effect

Fungal SMs have shown promising neuroprotective effects, offering potential therapeutic avenues for neurodegenerative diseases. These metabolites, derived from various fungi, exhibit diverse mechanisms of action, including antioxidant activity, modulation of signaling pathways, and inhibition of apoptosis. Several fungal SMs have neuroprotective effects. To date, several fungal metabolites have been used as neuroprotective drugs. These metabolites exhibit neuroprotection g two ways, one by oxidative stress reduction and one by reactive oxygen species (ROS) scavenging.

In oxidative stress reduction, the triterpenoid 2α-hydroxy-inotodiol (2α-HI) from the mushroom *Inonotus obliquus* has been shown to mitigate oxidative stress and apoptosis in SH-SY5Y cells by activating the Nrf2 and BDNF/TrkB/ERK/CREB signaling pathways. This suggests its potential as a dietary supplement for neurodegenerative diseases [191]. Another method is ROS scavenging, and to date, several metabolites from marine fungi, such as a melatonin analog from *Penicillium* sp., have demonstrated neuroprotective effects in Parkinson's disease models by scavenging ROS, thereby protecting neuronal cells from oxidative damage [192].

Some of the endophytic fungi have shown neuroprotective effects by producing polyphenols that can modulate multiple therapeutic targets, offering neuroprotection against disorders like Alzheimer's and Parkinson's disease. These compounds can weaken the complex physiology of neuronal disorders,

although detailed research on their bioactive compounds is limited [193]. In addition to this, metabolites from *Neosartorya fischeri* like Sartorypyrone E and other compounds have shown significant neuroprotective activity by inhibiting ROS accumulation and calcium ion influx, suggesting their potential role in neuroprotection related to their host plant, *Glehnia littoralis* [194].

Besides this, some of the fungi have been used to biotransform certain compounds into a metabolite effective for the treatment of neurological disorders. For instance, the biotransformation of cyclocephagenol by *Alternaria eureka* resulted in metabolites with potent neuroprotective activity. These metabolites reduced ROS levels and preserved mitochondrial integrity, highlighting the importance of structural modifications in enhancing neuroprotective effects [195].

Wu et al. isolated four compounds from the *M. purpureus* BCRC 38110 and characterized them for their structural conformation. Out of these 4 compounds, monascuspirolide B and ergosterol peroxide exhibited a concentration-dependent ability to attenuate paclitaxel-induced neurite damage in mouse dorsal root ganglion neurons. This suggests their potential as neuroprotective agents [189].

Kou et al. identified a new lanostane triterpenoid, 2α-hydroxy-inotodiol (2α-HI), from the edible mushroom *Inonotus obliquus*, which exhibited the most remarkable neuroprotective activity among 10 SMs tested against oxidative stress and apoptosis in SH-SY5Y cells. From the study, it was elucidated that the Nrf2 and BDNF/TrkB/ERK/CREB signaling pathways were involved in mediating these neuroprotective effects. Therefore, the application of fungal SMs, such as 2α-HI from *I. obliquus*, shows promise in potentially ameliorating neurodegenerative diseases through their neuroprotective properties [191].

Küçüksolak et al. explored the neuroprotective effects of fungal SMs derived from the biotransformation of cyclocephagenol using *Alternaria eureka*. Furthermore, the investigators discovered 21 new compounds with diverse structural changes that show strong neuroprotective effects against cell damage caused by H_2O_2. These metabolites demonstrated the capacity to decrease levels of reactive oxygen species (ROS) and maintain the integrity of the mitochondrial membrane, suggesting their possible use as neuroprotective agents. The study proposed that fungal SMs exhibit encouraging neuroprotective properties, underscoring their importance in prospective therapeutic uses for neuroprotection [195].

A team led by Bang investigated the neuroprotective properties of seminal membranes (SMs) generated by the endophytic fungus *Neosartorya fischeri* JS0553, which was obtained from *Glehnia littoralis*. Fischerin (compound 8) demonstrated notable neuroprotective effects on glutamate-induced HT22 cell death by blocking reactive oxygen species (ROS), calcium influx, and activation of mitogen-activated protein kinase. Moreover, the study indicated that fungal SMs, such as fischerin, have the capacity for neuroprotective uses, namely in reducing cell death caused by glutamate [194].

Yurchenko et al. discovered a novel melatonin analog, 6-hydroxy-N-acetyl-β-oxotryptamine (1), in *Penicillium* sp. KMM 4672 marine fungus. The isolated chemical protected against PQ-induced neurotoxicity and 6-OHDA-induced neuronal death better than melatonin. The metabolites scavenged ROS to protect the brain, with the novel melatonin counterpart being more effective. In 6-OHDA- and PQ-induced Parkinson's disease models, candidusin-related p-terphenyl polyketides' free radical scavenging and neuroprotective activities increased after C-3″ and C-4 dehydroxylation. So, fungal SMs may protect against Parkinson's disease models [192].

Fusarium lateritium SSF2's tricyclic pyridine alkaloid 4,6′-anhydrooxysporidinone was neuroprotective, according to Lee et al. This chemical protected hippocampus neurons against glutamate-induced oxidative stress and apoptosis. In glutamate-treated HT22 cells, 4,6′-anhydrooxysporidinone increased Nrf2 and HO-1 expression, blocked cytochrome c release, and cleaved caspase-9, -3, confirming its neuroprotective characteristics. Using fungal SMs like 4,6′-anhydrooxysporidinone may have neuroprotective effects [196].

According to Lee et al., edible-medicinal mushrooms can improve memory and cognitive functions and slow dementia and neurodegeneration in age-related neurodegenerative disorders, including Alzheimer's and Parkinson's. Mushrooms like *H. erinaceus*, *Ganoderma lucidum*, *Lignosus rhinocerotis*, *Pleurotus giganteus*, *Sarcodon scabrosus*, *Antrodia camphorata*, *Paxillus panuoides*, *Mycoleptodonoides aitchisonii*, and other species contain bioactive SMs that exhibit neuroprotective effects through mechanisms like anti-acetylcholinesterase activity, neurite outgrowth stimulation, nerve growth factor synthesis, enhancement of mitochondrial functions, reduction of endoplasmic reticulum stress, as well as antioxidant and anti-inflammatory effects [197].

Wu et al. isolated neuroprotective metabolites from the endophytic fungus *Penicillium citrinum* found in the mangrove *Bruguiera gymnorrhiza*. Two compounds, (*Z*)-7,4′-dimethoxy-6-hydroxy-aurone-4-O-β-glucopyranoside and (1*S*,3*R*,4*S*)-1-(4′-hydroxyl-phenyl)-

3,4-dihydro-3,4,5-trimethyl-1*H*-2-benzopyran-6,8-diol, were identified and shown to exhibit potent neuroprotective activity in oxidative damage-induced PC12 cells. Compound 1 exhibited potent neuroprotective activity in 1-methyl-4-phenylpyridinium-induced oxidative damage in PC12 cells. It was concluded that the fungal SMs, specifically those from *P. citrinum*, have a significant role in neuroprotection against oxidative stress-induced neurodegenerative conditions [198].

While the neuroprotective potential of fungal SMs is promising, further research is needed to obtain an depth understanding and their mechanisms and optimize their therapeutic applications. The diversity of fungal metabolites and their complex interactions with cellular pathways present both opportunities and challenges in developing effective neuroprotective agents.

7.2. Novel drug discovery from fungi

The potential of fungi as a source of novel drugs extends far beyond the antibiotics and anticancer agents already discovered. Advances in drug discovery technologies, including genomics, metabolomics [199], and synthetic biology are enabling researchers to explore previously untapped fungal species and their metabolites for new drug leads. The various ways for novel drug discovery from fungi are genome mining, endophytic fungi, marine fungi, synthetic biology, and fungal engineering.

7.2.1. Genome mining

Sequencing fungal genomes has revealed that many species possess cryptic or "silent" BGC that are not expressed under standard laboratory conditions. These clusters encode enzymes capable of producing novel metabolites with potential therapeutic applications. Genome mining, coupled with the activation of these silent clusters through environmental or genetic manipulation, is opening up new opportunities for drug discovery.

7.2.2. Endophytic fungi

Fungi that live within plants, known as endophytic fungi, have emerged as a rich source of bioactive compounds. Many of these fungi produce metabolites that mimic the pharmacologically active compounds found in their plant hosts, such as taxanes, alkaloids, and terpenes. Endophytic fungi represent an untapped reservoir for discovering new drugs with anticancer, antimicrobial, and anti-inflammatory properties.

7.2.3. Marine fungi

Marine fungi, which thrive in unique and often extreme environments, produce a diverse array of SMs not found in terrestrial fungi. These metabolites have shown promising activity against cancer, bacterial infections, and viral diseases. The exploration of marine fungi and their metabolites is an exciting frontier in the search for novel therapeutics.

7.2.4. Synthetic biology and fungal engineering

Synthetic biology approaches are being applied to engineer fungi for optimized metabolite production or the biosynthesis of entirely new compounds. By designing and assembling synthetic gene clusters, researchers can produce novel metabolites that may have enhanced bioactivity or reduced toxicity compared to natural products. Table 5 showcases important fungal SMs and their critical roles in medicine and pharmaceuticals, ranging from antibiotics and immunosuppressants to anticancer and antifungal drugs.

It is evident that fungi are a valuable source of bioactive compounds that are found in every field of science. The information gained related to the fungal SMs as an antibiotic, anticancer agents, and immunosuppressants has drastically transformed the healthcare sectors. Ongoing research in fungal genomics, endophytic fungi, and synthetic biology holds great promise for the formulation of new drugs that can address unmet medical needs in the future.

7.3. Applications of fungal secondary metabolites in industry and environmental biotechnology

Fungal metabolites have found widespread applications beyond medicine and agriculture, playing an essential role in various industrial processes and environmental biotechnology. From aiding in environmental cleanup to providing sustainable alternatives in manufacturing, fungi are a versatile and valuable resource. Here their applications in bioremediation, enzyme production, biofuels, and their use in natural dyes, flavors, and fragrances have been explained briefly.

7.3.1. Fungal metabolites in bioremediation and environmental sustainability

Fungal metabolites are essential to bioremediation, which uses organisms to break down contaminants. Fungi's robust enzymatic systems and capacity to adapt to severe environments make them a sustainable way to manage heavy metals, polymers, and hazardous chemical compounds.

Table 5. Applications of fungal secondary metabolites in medicine and pharmaceuticals.

Fungal SMs	Source fungus	Application	Medical/pharmaceutical benefits	References
2α-Hydroxy-inotodiol (2α-HI)	*Inonotus obliquus*	Neuroprotective	Oxidative stress reduction, Nrf2, and BDNF/TrkB/ERK/CREB signaling pathway activation	[191]
Aflatoxins	*Aspergillus flavus*, *A. parasiticus*	Research tool in oncology	Though toxic, aflatoxins are used in cancer research to study liver carcinogenesis and detoxification processes	
Brefeldin A	*Penicillium brefeldianum*	Antiviral and immunosuppressant agents	Exhibits antiviral activity and is used in immunological research for its effects on protein transport within cells	[200,201]
Cephalosporins	*Acremonium chrysogenum*	Antibiotic	Treats bacterial infections like bronchitis, pneumonia, and skin infections, similar to penicillin but broader	[202]
Cordycepin	*Cordyceps militaris*	Anticancer and anti-inflammatory agent	Shows potential in cancer therapy by inhibiting RNA synthesis and demonstrates anti-inflammatory and antioxidant properties	[203]
Cyclocephagenol metabolites	*Alternaria eureka*	Neuroprotective	Reduction of ROS levels, preservation of mitochondrial integrity	[195]
Cyclosporin A	*Tolypocladium inflatum*	Immunosuppressant	Used to prevent organ transplant rejection by suppressing the immune response	[174]
Echinocandins	*Glarea lozoyensis*, *Aspergillus nidulans*	Antifungal drug	Inhibits fungal cell wall synthesis, mainly treat systemic fungal infections like candidiasis and aspergillosis	[204,205]
Ergosterol	*Saccharomyces cerevisiae*	Precursor for steroid drugs	Used in the synthesis of vitamin D and corticosteroid drugs, beneficial for treating inflammation and immune disorders	[206]
Ergotamine	*Claviceps purpurea*	Vasoconstrictor	Used in the treatment of migraines and cluster headaches by constricting blood vessels	[207]
Fumagillin	*Aspergillus fumigatus*	Antiprotozoal, anticancer	Effective against microsporidiosis and used in cancer therapy to inhibit angiogenesis (new blood vessel formation)	[208]
Griseofulvin	*Penicillium griseofulvum*	Antifungal agent	Treats fungal infections of the skin, hair, and nails, such as athlete's foot and ringworm	[204,205]
Lovastatin	*Aspergillus terreus*	Cholesterol-lowering drug	Reduces blood cholesterol levels by inhibiting HMG-CoA reductase, a key enzyme in cholesterol synthesis	[209,210]
Melatonin analog	*Penicillium* sp.	Neuroprotective	ROS scavenging, protection against PD	[192]
Monapurpureusone, monascuspirolide B	*Monascus purpureus* BCRC 38110	Anti inflammatory	Anti-inflammatory effects, inhibition of IL-8 and TNF-α release in human chondrosarcoma cells, Attenuation of paclitaxel-induced neurite damage	[189,211]
Monaspin B	*M. purpureus, A. oryzae*	Antileukemic agent, anti-inflammatory, neuroprotective	Potent antiproliferative activity against HL-60 cell line	[190]
Mycophenolic acid	*Penicillium brevicompactum*	Immunosuppressant	Prevents rejection in organ transplantation and treats autoimmune diseases by inhibiting T and B cell proliferation	[212]
Paclitaxel (Taxol)	*Taxomyces andreanae*	Anticancer drug	Widely used in chemotherapy for cancers like breast, ovarian, and lung cancer by stabilizing microtubules	[213]
Penicillin	*Penicillium notatum*, *P. chrysogenum*	Antibiotic	Broad-spectrum antibiotics are effective against bacterial infections like pneumonia, sepsis, and syphilis	[176]
Sartorypyrone E	*Neosartorya fischeri*	Neuroprotective	Inhibition of ROS accumulation and calcium ion influx	[194]
Statins (Monacolin K)	*Aspergillus terreus, M. purpureus*	Cholesterol-lowering drug	Cholesterol-lowering drugs (HMG-CoA reductase inhibition), treatment of hypercholesterolemia	[187]
Strobilurins	*Strobilurus tenacellus*	Antifungal agent	Potential use in pharmaceuticals to develop new antifungal therapies	[214]

7.3.1.1. Mycoremediation of heavy metals. Fungi use their particular abilities (SMs) to convert, immobilize, and remove heavy metals from the environment in mycoremediation. This biotechnological approach is gaining attention due to its cost-effectiveness and environmental benefits compared to traditional methods. Fungal organisms have the ability to detoxify and eliminate heavy metals from contaminated soils and water. For instance, *A. niger* and *Trichoderma* spp. generate organic acids capable of binding and immobilizing heavy metals, such as lead, cadmium, and mercury, therefore inhibiting

their further dispersion in the environment. The absorption of heavy metals by fungi through their cell walls renders them a very suitable bioremediation agent for polluted habitats.

Primarily, mycoremediation occurs through two mechanisms: bioaccumulation and biosorption, and biotransformation and bio-oxidoreductases. Heavy metals can be accumulated and absorbed by fungi, especially filamentous species, through their cell walls and internal compartments. Fungal species exhibit distinct variations in the methods of bioaccumulation, biosorption, and biomineralization involved in this process [215,216].

Besides this, fungi can transform heavy metals into less toxic forms through biotransformation and bio-oxidoreduction processes. These mechanisms enhance the bioavailability of metals, facilitating their removal from contaminated sites [216,217].

Earlier investors have employed *A. niger* and *Candida albicans* for the remediation of heavy metals. These fungi have demonstrated significant efficacy in removing lead and cadmium from contaminated environments. In controlled studies, *A. niger* removed up to 85.6% of lead and 80% of cadmium, while *C. albicans* achieved similar results [218]. There are several investigations where fungi have been used in consortia with algae. The combination of fungi with microalgae, such as *Scenedesmus quadricauda*, enhances heavy metal removal efficiency. This synergy results in higher removal rates, with lead and cadmium reductions reaching up to 94 and 88%, respectively [218].

Studies have shown that the mycoremediation of pollutants is affected by various environmental factors. For instance, the efficiency of mycoremediation is influenced by factors, such as pH, temperature, and metal concentration. Optimal conditions vary depending on the fungal species and the specific heavy metals involved [215]. In addition to this, the extended period of exposure and the quantity of fungal biomass utilized are crucial factors. Experimental research has demonstrated that extended periods of exposure and increased amounts of biomass enhance the rates of heavy metal removal.

7.3.1.2. Degradation of persistent organic pollutants. Bioremediation with fungal SMs to degrade persistent organic pollutants (POPs) is promising. Due to their robust morphology and broad metabolic capacities, fungi help break down these contaminants. This method is cost-effective and environmentally friendly, making it suitable for large-scale environmental cleanups. Lignin-degrading enzymes like laccases and peroxidases in fungi break down POPs like PAHs and PCBs. White-rot fungi, such as *Phanerochaete* and *Pleurotus* species, break down hazardous compounds in soil and water, providing a sustainable alternative to chemical remediation.

SMs and oxidative enzymes from fungi break and degrade organic contaminants. These include laccase and cytochrome P450, which are essential for breakdown [219,220]. Fungal metabolisms degrade complex contaminants, including pharmaceuticals and polycyclic aromatic hydrocarbons, by oxidation, reactive intermediate generation, and chemical modification. Fungal enzymes, especially laccase, can oxidize several substrates into free radicals that are easier to break down. Using highly oxidative hydroxyl radicals, fungi destroy persistent contaminants, including antibiotics and endocrine disruptors [220]. Co-cultivating fungi and bacteria improves pollution breakdown. Fungal-bacterial co-cultures improve organic pollutant breakdown. These systems benefit from the synergistic interactions between fungi and bacteria, leading to more efficient pollutant breakdown. Co-cultures exhibit superior degradation capabilities for a range of contaminants, such as synthetic dyes and volatile organic compounds, relative to single-domain systems [221].

7.3.1.3. Plastic degradation. The utilization of fungal secondary metabolites (SMs) for the breakdown of plastics is a developing area that exploits the metabolic capacities of fungi to degrade durable plastic compositions. In addition to mitigating the environmental impact of plastic waste, this technology provides a sustainable means of recycling and upcycling polymers into useful compounds. Certain types of fungus possess the distinctive capability to break down complex plastics, such as polyurethane, polystyrene, and polyethylene. For example, *Aspergillus tubingensis* and *F. solani* catalyze the production of enzymes that have the ability to degrade plastic polymers, converting waste materials into biodegradable products. Fungal organisms synthesize a diverse range of enzymes, including laccases and peroxidases, that exhibit high efficacy in the degradation of plastics, such as polyethylene (PE) and polyvinyl chloride (PVC). Fungal enzymes commonly employed for lignin degradation have demonstrated encouraging outcomes in the degradation of resistant plastic polymers in controlled laboratory conditions [222]. More precisely, *Penicillium* and *Aspergillus* species have shown substantial activity of laccase and manganese peroxidase enzymes, which are essential for breaking down polypropylene (PP) [223].

Previous research has demonstrated that polyethylene can be converted into fungal SMs. This aerobic, catalytic digestion converts polyethylenes into

carboxylic diacids, which *A. nidulans* need for carbon. This fungus can then produce valuable SMs, such as asperbenzaldehyde, citreoviridin, and mutilin [224]. This method not only aids in plastic degradation but also expands the range of products derived from plastic waste, offering a dual benefit of waste reduction and resource generation [224]. Fungal strains, such as *Pyrenochaetopsis*, *Staphylotrichum*, and *Humicola* have been shown to degrade commercial bio-plastic films at ambient conditions. These fungi, through extracellular lipase activity, can hydrolyze ester bonds in bio-plastics, leading to significant degradation and transformation of the material. The use of fungal and bacterial consortia has been found to enhance the degradation process, achieving complete biodegradation of certain bio-plastic components within a month [225]. This fungal capability offers a promising solution to the global plastic pollution crisis.

7.3.1.4. Mycofiltration. Mycofiltration is a novel bioremediation approach that uses fungal mycelium to selectively remove and break down pollutants from water, providing a sustainable approach for treating wastewater. This technique could efficiently capture and kill pathogens, heavy metals, and other pollutants. Such techniques significantly increase the purity of water systems. From the literature, it is found that *A. niger* and *Fusarium proliferatum* uses their mycelial structures to absorb and break down organic contaminants. Various forms of this fungus, including mats and pellets, can be used to augment their efficacy in the removal of pollutants. Specifically, *A. niger* has demonstrated superior efficacy in eliminating organic contaminants, with pellets achieving a removal rate of up to 86.96% under ideal circumstances [226]. The ability of fungi to collect heavy metals in their biomass *via* processes, such as biotransformation and immobilization makes them highly efficient in the elimination of metallic pollutants from contaminated environments [216].

Mycofiltration has the potential to reduce the heavy metal content in the water which was evident from one of the studies where *Lentinus squarrosulus* reduced Pb, Cd, and Cr to undetectable levels in stormwater samples [227]. Fe (III) and imidacloprid were removed from the aqueous solutions by using *Pleurotus ostreatus* where the removal rates were achieved up to 94% for Fe (III) in column mycofiltration setups [228].

Mycelial pellets, due to their unique structure and surface properties, act as effective bio-carriers, which enhance the bioavailability and degradation of pollutants. These mycelial pellets can also support symbiotic relationships with other microorganisms, which maintains stable pollutant removal efficiency [229].

It is concluded that mycofiltration is an economical and eco-friendly alternative to traditional physicochemical methods, which are less efficient and costly [216]. Through these bioremediation techniques, fungal metabolites contribute significantly to environmental sustainability by helping to restore the environment affected by industrial pollutants.

7.4. Industrial enzymes and biofuels

Fungai is one of the major sources of industrial enzymes that find applications in food, textiles, detergents, paper industries, and biofuel production. Fungal enzymes, being highly efficient and biodegradable, offer sustainable alternatives to chemical catalysts.

7.4.1. Fungal enzymes

Fungi produce a wide variety of extracellular enzymes in large amounts, having low cost with suitable shelf life, and which can be purified easily by simple purification methods. Since ancient times, fungi have been used in a wide range of industries as enzymes, including baking, cheese production, the brewing industries, the production of antibiotics, manufacturing of linen and leather goods [230]. Enzymes are classified into seven different categories, which include oxidoreductases, transferases, lyases, hydrolases, isomerases, ligases, and translocases [231,232]. On the other hand, the hydrolases and oxidoreductases classes comprise the commercially significant enzymes. Although there are currently more than twenty recognized classes of oxidoreductases, the most studied classes of oxidoreductase include dehydrogenase (that transfers hydrogen atom to an electron acceptor), peroxidase (final electron acceptor is peroxides), and oxygenase (final electron acceptor is oxygen) [233].

Monooxygenase, dioxygenase, and laccases are the commercially used oxygenases. Laccases can oxidize both phenolic and non-phenolic compounds without any requirement of co-factor. The cofactor-independent nature of laccase nominated itself to various industrial applications, such as stain removal [234], the paper industry [235], biosensor manufacturing [236], and medical applications. Many peroxidase enzymes are defined as metal-dependent enzymes. The two most widely studied peroxidases include manganese peroxidases (MnP) and lignin peroxidases (LiP). Basidiomycetes, also called white-rot fungi can degrade lignin because they produce LiP and MnP extracellularly [237]. Furthermore,

the usage of fungal enzymes in food production is a smart way to extend the shelf life of food without sacrificing its nutritional content. One common fungal enzyme used in food applications is L-asparaginase. It is generally recognized as safe (GRAS) and used as a food additive to prevent the formation of acrylamide, which is generated when food ingredients react, including reducing sugars and amino acids at high temperatures and low humidity conditions [238].

Fungal enzymes are also used in green-environmental policies to make our environment clean and safe. The health and welfare of people are seriously threatened by environmental pollution as a result of the increasing human population. The usage of fungal enzymes to manage waste provides a green approach to the development of sustainable environments. Currently, polyethene-based materials are accumulating in our environment at a high rate due to their low cost and they also provide very high resistance to biological remediation techniques. To deal with this situation, molecular docking, and simulation studies have been conducted on different enzymes, including manganese-peroxidase, laccase, and lignin-peroxidase, to explore their roles in the eco-friendly degradation of polyethene-based materials [239]. Similarly, fungal laccases are also used in the bioremediation of synthetic textile dyes by transforming the dye molecules into safer, eco-friendly, and non-colored structures [240]. Fungal species, such as *Alternaria*, *Cladosporium*, and *Aspergillus* exhibit the ability to produce extracellular enzymes, such as lignin peroxidases, laccases, and manganese peroxidases which can effectively break down complex synthetic dyes like Indigo carmine, methyl green, Congo red, and Poly R-478 [241].

New government policies have been imposed on paper industries to avoid the use of chemical bleaching processes and encouraged them to develop eco-friendly alternative methods. Eco-friendly papermaking depends on two processes: biopulping followed by biobleaching. Biopulping refers to the pretreatment of agricultural waste pulp by lignin-degrading enzymes followed by biobleaching. The white rot fungi, including *Phlebia tremellosa*, *Phellinus pini*, *Ganoderma austral*, and *Ceriporiopsis subvermispora*, contain cellulose-free lignin-degrading enzymes required for the biopulping process. The white rot fungus, *Phanerochaete chrysosporium* has a complete enzymatic system for breaking down lignin [242]. Biobleaching is defined as the complete elimination of lignin from bio-pulping waste by using a fungal enzyme to produce the white, bright pulp required for paper making. In the biopulping process, xylanases enzymes isolated from *Talaromyces thermophilus* and *Fusarium equiseti* MF-3 have been used to reduce the usage of organo-chlorocompounds used in the conventional bleaching process [243,244].

7.4.1.1. Cellulases and xylanases. These enzymes are mainly produced by the *Trichoderma reesei* and *A. niger*, which find huge applications in the paper and pulp industry. These enzymes have to potential to break down complex molecules of the plants, which improves the quality of the paper and reduces the need for harsh chemicals. In addition to this, these enzymes are also essential in the production of biofuels, as these enzymes facilitate the breakdown of lignocellulosic biomass into fermentable sugars that can be transformed into ethanol [245].

7.4.1.2. Proteases and lipases. Fungal proteases find applications in the detergent and food sectors. Fungal proteases used for the tenderization of meat, beverage clarification, and fermentation are mainly derived from *A. oryzae* and are employed for the purpose of meat tenderization. Lipases, catalyze the breakdown of lipids and are employed in the synthesis of biodiesel, a sustainable substitute for petroleum-derived fuel [246].

7.4.1.3. Amylases. Fungal amylases, synthesized by the species *A. oryzae*, play a crucial role in the food and beverage sectors by enzymatically turning starch into sugars. They are used in brewing, baking, and the production of high-fructose corn syrup. Amylases also play a key role in bioethanol production, where they help in the breakdown of starches into fermentable sugars [247].

The use of fungal enzymes in these industries not only improves production efficiency but also reduces the environmental impact of traditional manufacturing processes, contributing to a more sustainable industrial ecosystem. Table 6 summarizes the fungal SMs and their industrial and environmental biotechnological applications.

7.5. Role of fungal SM as biofuels

Fungal SMs play a significant role in biofuel production due to their ability to break down complex organic materials and produce energy-rich compounds. Biodiesel and other liquid transportation fuels are made from fungi's metabolites. Fungal enzymes are essential for second-generation biofuels made from agricultural wastes and forest trash. Biofuel production relies on fungi to break down lignocellulosic biomass into simpler sugars that can be fermented into ethanol and butanol using cellulases and ligninases. Biofuel research is accelerating with genetically modified *Trichoderma* and *Aspergillus* species that produce more enzymes. The increasing

Table 6. Summarized fungal secondary metabolites and their industrial and environmental biotechnological applications.

Fungal SMs	Source fungus	Applications	Industrial/environmental benefit	References
Citric acid	*Aspergillus niger*	Food and beverage industry	Used as a natural preservative, flavor enhancer, and acidulant in foods and beverages.	[201]
Amylase	*Aspergillus oryzae*	Starch conversion in food processing	Enzyme used in brewing, baking, and production of high-fructose corn syrup by breaking down starches into sugars.	[248]
Cellulase	*Trichoderma reesei*	Biofuel production	Breaks down cellulose in plant biomass into fermentable sugars, enhancing the production of bioethanol and other biofuels.	[249]
Laccase	*Pleurotus ostreatus*	Bioremediation	Degrades pollutants, such as dyes, phenols, and other organic contaminants, aiding in wastewater treatment and soil remediation.	[250]
Pectinase	*Aspergillus niger*	Textile and paper industries	Used for retting fibers in textile production and for pulping in the paper industry by breaking down pectin in plant cell walls.	
Protease	*Aspergillus oryzae*	Detergent industry	Used in laundry detergents to break down protein-based stains, improving cleaning efficiency.	[251]
Fumaric acid	*Rhizopus oryzae*	Plastic and resin production	Used as a building block in the production of biodegradable plastics and as a precursor for alkyd resins.	
Gluconic acid	*Aspergillus niger*	Metal cleaning and textile industry	Acts as a chelating agent in metal cleaning and helps in textile processing by adjusting pH levels.	[252]
Itaconic acid	*Aspergillus terreus*	Polymer industry	Serves as a monomer for synthetic resins and biodegradable plastics, providing an eco-friendly alternative to petroleum-based products.	[253]
Tannase	*Aspergillus niger*	Beverage industry	Used in tea, coffee, and wine production to reduce bitterness and improve flavor.	[254]
Gibberellins	*Gibberella fujikuroi*	Agriculture and horticulture	Promotes plant growth, enhances crop yield and quality, particularly in fruit ripening and seed germination.	[255,256]
Penicillin G amidase	*Penicillium chrysogenum*	Pharmaceutical industry	Facilitates the synthesis of β-lactam antibiotics like penicillin and amoxicillin by catalyzing selective reactions	[10,257]
Aflatrem	*Aspergillus flavus*	Pest control	Used in developing eco-friendly biopesticides to control insect pests in crops	[258]
Biosurfactants	*Candida bombicola*	Oil recovery and environmental cleanup	Reduces surface tension in oil spills and enhances oil recovery from petroleum reservoirs, aiding in environmental cleanup efforts	[259]
Chitinase	*Trichoderma harzianum*	Waste management and agriculture	Degrades chitin in shellfish waste, recycling waste, and controls fungal pathogens in plants as a biocontrol agent	[260]

availability of fungal genomic data facilitates the identification of metabolic pathways for hydrocarbon production, expanding the potential of fungal-derived compounds in the biofuel industry [261].

Oleaginous fungi like *A. niger* can convert palm oil mill effluent (POME) into lipids that can be trans-esterified into biodiesel. This dual-benefit approach produces biodiesel and bio-remediates industrial waste [262]. Oleaginous mushrooms can accumulate up to 70% of their dry weight in lipids, making them an efficient biodiesel source. Conventional transesterification converts these lipids into biodiesel, a possible third-generation biofuel feedstock [263,264]. Several oleaginous species accumulate lipids that can be converted into biodiesel precursors like fatty acid methyl esters (FAME).

Additionally, fungi like *S. cerevisiae* produce ethanol through fermentation and can be optimized to produce higher-energy alcohols like butanol. Some of the studies genetically manipulated the *S. cerevisiae* and obtained higher biofuel. Daniel et al. exhibited that metabolically engineered *S. cerevisiae* can boost biofuel output like ethanol and fatty acid ethyl esters (FAEEs). This involves providing fungi lignocellulose-degrading enzymes to use non-food biomass for biofuel generation [265]. Advancements in genetic modification have enabled the enhanced synthesis of SMs in fungi, which can serve as precursors for biofuels. This involves the genetic manipulation of specific genes to enhance the synthesis of targeted metabolites [265].

The above section revealed that fungi produce a diverse range of SMs, which may also promise sustainable sources of transportation fuels. Chemicals, such as limonene and sesquiterpenes can be enhanced to function as components of high-energy fuels like jet fuel and diesel [261]. Fungi may also contribute to the production of biogas by assisting in the anaerobic breakdown of organic waste, which leads to methane production for energy purposes. Table 7

Table 7. Summarizes some fungi and their secondary metabolites that are relevant to biofuel production.

Fungui candidate	Secondary metabolite	Role in biofuel production	References
Aspergillus niger	Citric acid	Used in the fermentation process to enhance sugar conversion.	[266]
Aspergillus oryzae	Glucose oxidase	Converts glucose into gluconic acid, improving fermentation yield.	[248]
Saccharomyces cerevisiae	Ethanol	Primary yeast for ethanol production from carbohydrates.	[267]
Fusarium oxysporum	Mycotoxins (e.g., fusaric acid)	May impact the fermentation process; its role is complex.	[268]
Penicillium chrysogenum	Penicillin	Not directly used in biofuel but affects fungal biomass quality.	
Neurospora crassa	Enzymes (e.g., cellulases)	Breaks down complex carbohydrates into fermentable sugars.	[269]
Candida albicans	Various enzymes	Helps in converting sugars into ethanol, improving yields.	[270]
Cladosporium spp.	Various metabolites	Influences lignocellulosic biomass degradation.	[271]

summarizes the effects of several fungi and their metabolites on several phases of biofuel production, ranging from improving fermentation processes to decomposing biomass.

7.6. Fungal secondary metabolites as natural dyes, flavors, and fragrances

7.6.1. Pigment and colorants

The worldwide demand for colorants is increasing rapidly in the field of food, cosmetic, and textile industries. Natural colorants have been discovered as alternatives to synthetic colorants because of their deleterious effects on the environment and consumers. With this, synthetic food colorants exhibit negative effects on human health, including cancer, neurological disorders, asthma, and allergies. Pigments derived from microorganisms show several advantages, such as increased yield, cost-effectiveness, stability, labor cost, supply sustainability, and ease of downstream processing. Fungal metabolites have emerged as sustainable alternatives in the production of natural dyes, flavors, and fragrances. These metabolites offer eco-friendly and nontoxic substitutes for synthetic chemicals commonly used in the textile, food, and cosmetic industries.

The appropriate use of the fermentation physiology of fungi, together with genetic engineering tools, can lead to massive production of pigments from fungi. Pigments obtained are utilized in cosmetic industries as colorants, textiles, food, beverages, pharmaceuticals, additives, antioxidants, and color intensifiers [272]. Concerning its detrimental effects on the environment and public health, the textile dying industry is thought to be the most polluting segment of the fashion industry. Many fungal genera/species are used at commercial scale, to produce pigments and colorants.

Pigments from fungi, like polyketides, melanin, and carotenoids, are widely used as natural dyes in the textile and food industries. For instance. *Monascus* spp. produces red, yellow, and orange pigments that are used as food colorants. *Aspergillus* and *Penicillium* spp. also produce vibrant pigments with potential applications in the dyeing of the fabric. Such myco-derived dyes have several advantages, like biodegradable and nontoxic, and eco-friendly in comparison to their synthetic dyes.

The ability of four major fungi to produce a diverse range of pigments includes *Emericella purpurea* (red pigment producer), *Paecilomyces marquandii* (yellow pigment producer), *Talaromyces albobiverticillius* (deep red pigment producer), and *Trichoderma harzianum* (yellow-brown pigment producer) was studied by Lebeau et al. [273]. Fungal pigments are classified into different types of chemical classes, including phenazines, quinones, carotenoids, flavins, monascin, indigo, azophilones, melanins, and violacein. Some *Monascus* species, including *M. purpureus* and *M. ruber*, are used as food additives and colorants because they can synthesize red pigment [274]. The pigments derived from *M. ruber* are named monarubrin, rubropunctin, N-glucosylrubropunctamine, and N-glucosylmonascorubramine. Pigments are categorized into six distinct groups based on their color: (1) yellow pigment (monascin, $C_{21}H_{25}O_5$, and ankaflavin $C_{23}H_{30}O_5$), (2) orange pigment (rubropunctatin, $C_{21}H_{22}O_5$, and monascorubrin, $C_{23}H_{26}O_5$), and (3) red pigment (rubropunctamin, $C_{21}H_{26}NO4$, and monascurubramin $C_{23}H_{27}NO_4$) (Figure 10). The pigment composition of *Monascus* species is influenced by several parameters, such as nitrogen source, substrate chemistry, temperature, pH, and agitation [275]. Additionally, Viggiano et al. reported that *P. chrysogenum* produces a yellow pigment known as chrysotile [276]. Many of the pigments obtained from fungi are used as food colorants; for example, riboflaAbvin from *Ashbya gossypii*, janthinorubrin, a pink-red from *P. oxalicum*, β-carotene from *Blakeslea transport*/*Phycomyces blakesleeanus*/*Mucor circinelloides*, and Monascus pigments from *M. purpureus* is used as food colorants at commercial level [272]. Numerous studies have shown the wide range of uses for fungal-produced pigments in the textile industry. Because of their high stability and good color fastness to washing, a variety of pigments, including yellow pigment from *S. ganodermophthorum*, red pigment from *S. cuboideum*, and green pigment from *C. aeruginosa* are used for

Figure 10. Chemical structure of pigments formed by *Monascus purpureus*. (A) Rubropunctamin; (B) Monascorubramin; (C) Rubropunctatin; (D) Monascorubrin; (E) Monascin; (F) Ankflavin.

dyeing purposes on bleached cotton, spun polyacrylic, spun polyamide (nylon 6.6), worsted wool, spun polyester (Dacron 54) and garment fabrics.

7.6.1.1. Flavor compounds. Besides this, various fungal compounds are used as flavor compounds. For instance, fungi are known for their ability to produce a wide range of volatile organic compounds (VOCs) that contribute to the flavor and aroma of foods. *Penicillium roqueforti* and *Penicillium camemberti* play a crucial role in the production of blue and white cheeses by providing distinctive flavors and textures. Furthermore, some of the fungi are also involved in the fermentation of various foods and beverages, which enhances the flavor and texture of the final product. One well-known flavor enhancer produced by fungus during fermentation is citric acid, which finds applications in sauces and other drinks.

7.6.1.2. Fragrances. Several fungi produce aromatic SMs, which could be used in the fragrance and cosmetic industries. For instance, certain fungi like *Cladosporium* and *Aspergillus* produce terpenes, alcohols, and esters, which have been used in perfumes and essential oils. Nowadays, such fungal SMs could be used as the best alternative to synthetic chemicals, which are mainly derived from petroleum sources.

The application of fungi SMs for all the above purposes not only decreases environmental pollution but also meets the growing consumer demand for eco-friendly and organic products. The enzymes and metabolites produced by fungi play a significant role in bioremediation, industrial processes, and the production of natural products. With more innovative approaches in this field, it is possible to provide eco-friendly and economical alternatives to traditional approaches. Table 8 presents a summary of different fungal metabolites together with their inherent characteristics as dyes and pigments.

7.6.2. Role of fungi and their secondary metabolites in food industries

Fungal SMs play a significant role in the food industries as nutritional supplements, preservatives, and enzymes for various food and beverage industries. There are several organic acids produced by fungi, like citric acid and lactic acid, which act as food preservatives by preventing the growth of microorganisms. Besides this, some of the fungi also produce alcohols, esters, and terpenes, which enhance the taste and flavor of fermented food and beverages, like cheese, soy sauce, and beer. Furthermore, some of the fungi produce natural pigments like anthraquinones and polyketides, which are used as natural food colorants. These natural food colorants are a sustainable and eco-friendly alternative to synthetic colors used in the food processing industry. Moreover, fungi produce various enzymes like amylases, proteases, and lipases, which find applications not only in foods and beverages but also in the baking, brewing, and dairy industries. To enhance the shelf life of meat and dairy products, natamycin, a fungal SM, is widely used as a preservative. Nonetheless, fungi also produce several vitamins, antioxidants, and bioactive compounds that are used

Table 8. Fungal secondary metabolites used as colorants, and pigments in the industries.

Fungus candidate	Secondary metabolites	Application	References
Monascus purpureus	Monascorubrin, rubropunctatin	Natural red and orange pigments are used as food colorants (e.g., in rice)	[275]
Penicillium species	Patulin, penicillic acid	Contribute to complex flavors in fermented foods like cheese	[277]
Aspergillus niger	Citric acid	Used as a flavor enhancer in food and beverages	[278]
Cladosporium cladosporioides	Cladosporin	Yellow pigment with potential application in textile dyeing	[279]
Fusarium solani	Naphthoquinones (e.g., fusarubin)	Produces red pigments for potential use in fabric dyes	[280]
Trichoderma viride	Trichodermin	Adds earthy aromas to foods and beverages.	[281]
Aspergillus oryzae	Kojic acid	It is used in cosmetic formulations for skin brightening, but it also has the potential to create natural fragrances	[282]
Pleurotus ostreatus	Laccase enzyme	Potential in natural dye production by oxidation of plant-based compounds	[283]
Eurotium amstelodami	Eurotinone	Produces yellow pigments for use as dyes in food or textiles.	[284]

in functional foods and dietary supplements to enhance their nutritional value [285,286].

7.6.2.1. Edible mushrooms: To improve human health. Basidiomycetes is a major class of fungi, able to grow at different environmental conditions and leads to the creation of a range of SMs. As opposed to ascomycetes fungi, basidiomycetes exhibit a diverse nature in their morphology and SMs. The majority of edible basidiomycetes reproduce in the roots of their host plant. Although it is very challenging to determine the exact number of species that make up the basidiomycetes, reports indicate that 14,000 species of mushrooms are identified as basidiomycetes, out of which 7000 spp. are edible and over 2000 spp. are high-value edible species of mushrooms [287]. Currently, there are more than 100 species of mushrooms that can be cultivated. *Agaricus bisporus*, *Lentinus edodes*, *Flammulina velutipes*, and *Pleurotus* spp., are recognized as the most cultivable mushroom worldwide. Due to their higher nutritional content, edible mushrooms have been used for a longer time [288,289]. With this, they are also used as nutraceuticals and pharmaceuticals to treat a range of chronic illnesses. They are regarded as significant sources of fats, protein, and various minerals, including iron (Fe), copper (Cu), manganese (Mn), and vitamins B1, B2, B12, C, D, and E (Figure 11) [289].

Over the past ten years, medicinal mushrooms have gathered a lot of attention. It has been proposed that these mushrooms are micro factories of the pharmaceutical industry, that are responsible for the synthesis of bioactive compounds. The bioactive compounds derived from edible mushrooms belong to different classes, such as glycosides, volatile oils, lectins, alkaloids, terpenoids, polysaccharides, flavonoids tocopherols, carotenoids, phenolics, folates, vitamins, minerals, and organic acids [290]. In edible mushrooms, the type and quantity of bioactive compounds vary depending on the strain, storage conditions, age, cultivation, processing, and cooking techniques [291]. The predominant polysaccharide present in mushrooms is β-glucans, which also make up half of the mass of fungal cell walls. β-glucans show anticancer, anti-oxidant, neuroprotective, anticholesterolemic, and immunomodulating activities. The mushroom produces a wide range of peptides and proteins called ribosome-inactivating proteins [292], ribonucleases, laccases, and antimicrobial and immunomodulatory proteins [293]. The most extensively grown and edible mushroom worldwide is the white common mushroom, *A. bisporus*. Lectin and proteins from *A. bisporus* and polytricha are immune stimulants. According to Latif et al., *A. bisporus* inhibits the multiplication of breast cancer cells driven by 7,12-dimethylbenz[*a*] anthracene (DMBA) in rats. *Agaricus blazei* is also an antioxidant, antidiabetic, and hepatoprotective [294]. Traditional mulberry-growing fungus *Sanghuangporus sanghuang* treats inflammation [295]. Isolated triterpenoids from *S. sanghuang* fungus mycelium show antioxidant activity against hydroxyl radicals, ABTS, and DPPH free radicals [296]. Eating edible fungi can alleviate serious diseases like diabetes and obesity. A recent study has reported that the administration of *Pleurotus citrinopileatus* water extracts to obese mice can significantly lower the mice's weight, triglycerides, low-density lipoprotein (LDP), and cholesterol [297,298]. The ascomycete fungi known as *Eurotium cristatum* lessen the effects of obesity in mice by controlling the stomach flora of mice [298].

7.6.2.2. Mycoprotein: Fungal protein for human consumption. Mycoprotein refers to a protein-rich diet derived from fungal biomass that can be consumed as a substitute for meat. Currently, supermarkets in industrialized nations mostly offer mycoprotein in the form of sausages and patties [299]. Food items that can replace meat should offer comparable nutritional advantages, and those that can fulfill this criterion are called meat analogs, meat alternatives, meat replacers, or meat substitutes [300]. These food items can be obtained from plants (soy, pea, oat), and animals (milk) or can be microbe-

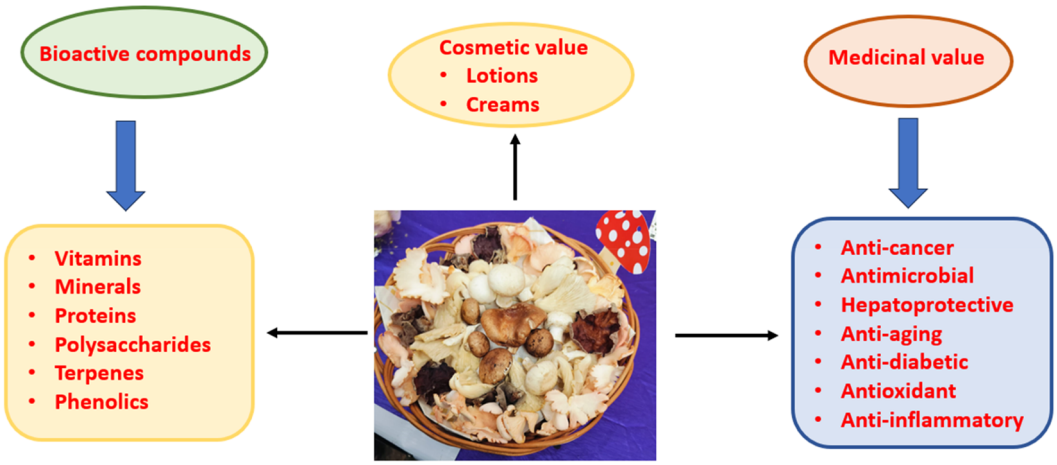

Figure 11. Schematic representation of therapeutic properties and bioactive compounds of mushrooms.

derived products (mycoprotein). In Europe, both *P. roquefortii* and *P. camembertii* are widely used to make blue (Roquefort, Gorgonzola) and soft-ripened (Camembert and Brie) cheese. As a result, mycoprotein, a food made from fungal filamentous biomass, can be processed and utilized. The Food and Drug Administration (FDA) in the United States (US) has designated mycoprotein as GRAS since 2002 [301]. A full protocol for the synthesis of mycoprotein has been described by Finnigan et al. [302]. Mycoprotein is defined as a high protein, low fat, and high-fiber food component. However, there are certain issues because of its high RNA content. Mycoprotein from *Fusarium venenatum* is produced by heating the fungal biomass in liquid broth to a temperature above 60 °C for 20–45 min, which reduces the amount of RNA by 2%. Mycoprotein fiber composition consists of 2/3rd of beta-chitin and one-third chitin, forming a fibrous chitin-glucan matrix that is 80% insoluble in water. Ingestion of the chitin component helps to relieve pain occurring from osteoarthritis and promotes the growth of healthy bacteria in the colon (Figure 12). The consumption of mycoprotein improves the body's glycemic profile and lowers cholesterol levels in the body [303,304]. The administration of 88 g of wet-weight mycoprotein per day can significantly reduce blood cholesterol levels, as shown by a study [305,306].

7.6.2.3. Saccharomyces boulardii: A successfully used probiotic. Probiotics are defined as living organisms, that improve human health when ingested in proper amounts. Additionally, it is believed that they should not impart any pathogenicity, toxicity, or genes for antibiotic resistance gene to the host. Probiotics are usually consumed in the form of fermented foods which contain bacteria that produce lactic acids, such as *Streptococcus* spp., *Bacillus* spp., *Bifidobacterium* spp., *Enterococcus* spp., and *Lactobacillus* spp., which are generally used to cure patients suffering from gastrointestinal disorders [307]. However, the characteristics of probiotics vary greatly among species, strains, and even between strain variants, indicating that the benefits they offer are strain/variant specific [307,308]. Although several bacteria have been identified as probiotics, many yeast species represent probiotics properties. The commonly used baker's yeast *S. cerevisiae* does not provide any health benefit to humans, but its close relative *S. boulardii* is generally used to treat acute diseases like diarrhea and chronic inflammatory bowel disease (IBD). Till now, this is the only yeast that is currently used as a probiotic; the Biocodex Laboratories strain of *S. boulardii* CNCM I-745, also called *S. boulardii* Hansen CBS 5926, is a major example of the prebiotic qualities of this yeast and has been the focus of more than 80 randomized clinical trials [309]. Probiotics must be able to endure the harsh environmental conditions of the gastrointestinal tract. Probiotic formulations containing *S. boulardii*, *Lactobacillus* spp., and *Bifidobacterium* spp. were tested *in vitro* which indicates that *S. boulardii* has greater capacity to survive under GI tract conditions. *Saccharomyces boulardii* is also able to thrive in an intestinal environment that includes bile salts and pancreatic enzymes [310]. Probiotics exhibit numerous mechanisms, such as regulation of the gut microbiome, pathogen competition, metabolic control, immune system control, interaction with the brain-gut axis, mucin production, and cellular adhesion [307,309].

The use of probiotics is generally recommended to reestablish the healthy gut microflora upon dysbiosis. Gut dysbiosis is defined as the change in the composition of the host microbiome, both quantitative and qualitative. These changes are associated with various diseases leading to antibiotic-associated diarrhea, acute infectious diarrhea, and IBD [311]. Treatment with probiotics helps to maintain gut-associated microbial community. Probiotics

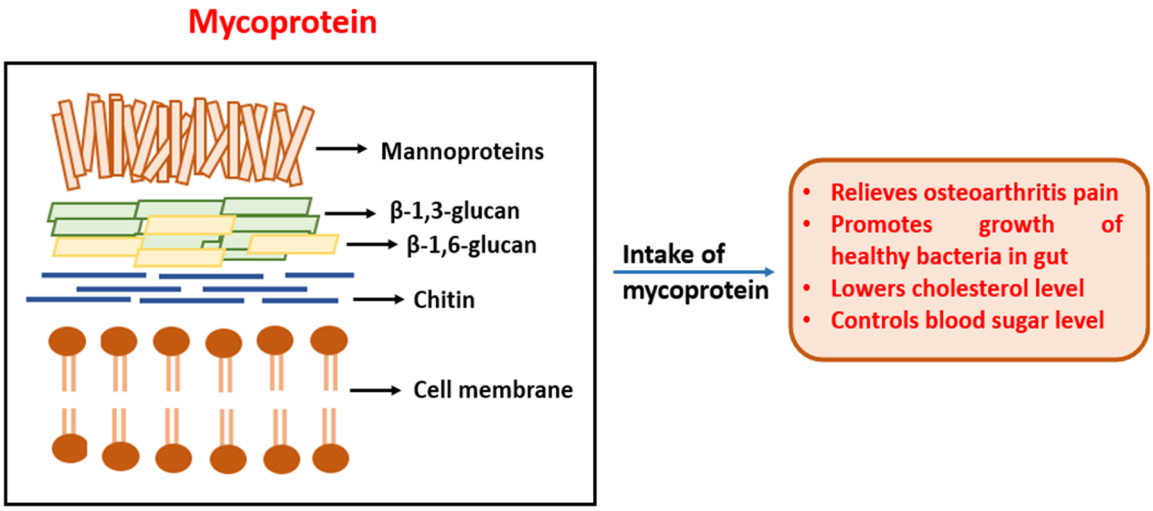

Figure 12. Therapeutic properties of mycoprotein.

exhibit antimicrobial activity against pathogenic microorganisms. The production of inhibitory compounds and modulation of signaling pathways can achieve antagonism against pathogens. *Saccharomyces boulardii* secretes antimicrobial peptides [312] that decrease the adhesion of *Citrobacter rodentium* to host epithelial cells by altering virulence factors [308]. It also secretes 54-, 63-, and 120-kDa protein that cleaves out various microbial toxin proteins. *Saccharomyces boulardii* produces 54-kDa serine protease, which cleaves toxins A and B of *C. difficile* and the enterocytic receptor to which toxins bind and leads to inflammation, fluid secretion, mucosal permeability, and injury in the intestines [313]. Another study demonstrated the ability of *S. boulardii* to inhibit *Escherichia coli* surface endotoxins by dephosphorylation. *Saccharomyces boulardii* produces 63-kDa alkaline phosphatase that targets the lipopolysaccharides (LPS) and contributes to decreased synthesis of tumor necrosis factor (TNF-α) [314]. *Saccharomyces boulardii* is also used in the treatment of the Gram-negative bacterium *Helicobacter pylori* responsible for causing chronic gastritis and duodenal cancer [315].

Saccharomyces boulardii strain CNCM I-745 provides defense against lethal toxins produced by *Bacillus anthracis*. The bacteria use a toxin to adhere to the intestinal epithelium, causing ulcerative lesions in the jejunum and cecum of the intestine that result in bleeding and the formation of ulcers [316]. *Saccharomyces boulardii* provides a protective effect by producing protease enzymes to degrade this lethal toxin. Additionally, in contrast to bacterial probiotics like *Lactobacillus* spp., *S. boulardii* does not transfer any genes that confer antibiotic resistance. Clinical trials have been conducted to assess the efficiency and safety of *S. boulardii*. This yeast was believed to improve several diarrheal illnesses including pediatric diarrhea, acute diarrhea, acute traveler's diarrhea caused by bacterial, viral, or parasites, and antibiotic-associated diarrhea [309].

7.6.2.4. Fatty acid production from oleaginous fungi. Oleaginous fungi can accumulate lipids, which make up 20–80% of their dry biomass. These lipids are also called microbial lipids or single-cell oils (SCOs). They are recognized as lipid-producing microorganisms because of their very short growth cycle, high yield of lipids, and capacity to produce large amounts of biomass. In recent years, microbial lipids derived from oleaginous fungi have gathered more attention because of their potential application in biodiesel production, medicine, and food supplements [262]. The majority of lipids are composed of fatty acids, which are made up of long hydrophobic carbon chains that are terminated with polar carboxylic acid groups. Of all the fatty acids, polyunsaturated fatty acids (PUFAs) are essential because they are not made by the human body and, therefore, taken from the diet [317]. Fish and plant seeds are two common and traditional sources of PUFAs, but their yield is very low. So, keeping in mind about higher demands of PUFAs, microbes, such as algae and fungi are considered as major platforms for the alternative production of PUFAs. Many oleaginous fungi including *Galactomyces geotrichum*, *M. circinelloides*, and *Mortierella isabellina* have been studied for their ability to accumulate polyunsaturated fatty acids (PUFAs) with their potential use [318]. PUFAs play a crucial role in the human body, and they belong to ω-3 and ω-6 classes, also defined as precursors of eicosanoids, a structural element of membrane phospholipids. PUFAs are defined as long-chain fatty acids containing two or more double bonds, including linoleic acid gamma-linolenic acid (GLA), (LA), conjugated linoleic acid (CLA), docosahexaenoic acid (DHA), eicosapentaenoic

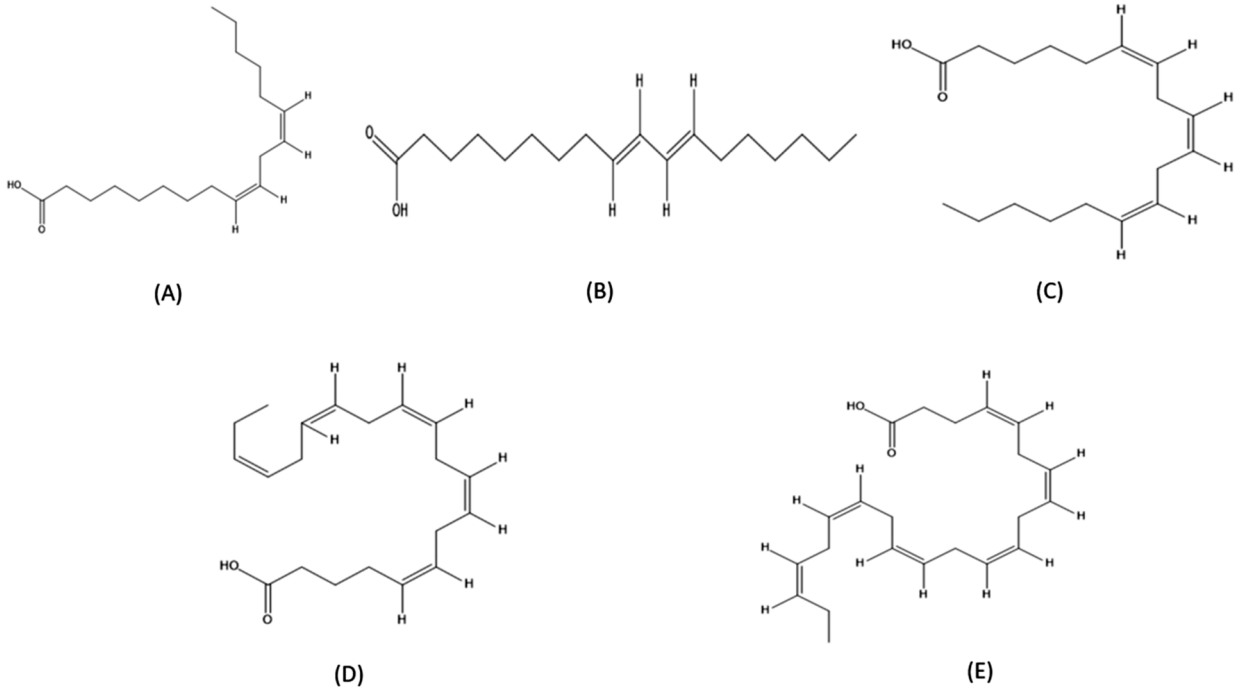

Figure 13. Chemical structures of (A) Linoleic acid (LA); (B) Conjugated linoleic acid (CLA); (C) Gamma-linolenic acid (GLA); (D) Eicosapentaenoic acid (EPA); (E) Docosahexaenoic acid (DHA).

acid (EPA). These fatty acids also serve as building blocks of bioactive molecules (Figure 13).

Each of them exhibits biological activity that includes anti-aging, anti-inflammatory, anti-oxidative, and anti-tumor. *Mucor circinelloides* is defined as a model oleaginous fungus in the study of fatty acid synthesis and lipid accumulation and production of other important bioactive compounds (Figure 14) [317].

In the middle of the 1990s, the United Kingdom (UK) acknowledged it as the first organism capable of producing gamma-linolenic acid (GLA; 18:3, delta-6,9,12) at a commercial level [319]. With the aid of genetic engineering tools, *M. circinelloides* have been modified to produce a substantial amount of biotechnologically important precursors for the food and pharmaceutical industries. GLA has several advantages in the field of health to treat various diseases, such as inflammatory disorders, cancer, cardiovascular-associated disease, and diabetes [320]. Besides this, *M. circinelloides*, can degrade a broad range of organic pollutants, like dyes, phenolics, and polycyclic aromatic hydrocarbons. This is achieved through the production of enzymes and metabolites that break down these complex compounds into less toxic forms [321,322]. Among all, oil-producing filamentous fungi, *M. circinelloides* is considered an essential organism for studying lipid accumulation because of its ability to make lipids rich in GLA [317]. According to research studies, *M. circinelloides* contained 19, 11, and 38% of GLA, linoleic acid, and oleic acid. The proportion of GLA can be increased by overexpression of two delta-6 (delta6-2 and delta6-1), and delta-12 desaturases in *M. circinelloides*. Here, GLA yield and GLA in total fatty acids increased by up to 33 and 38% in comparison to the control strain. This also suggests that delta-6 desaturase is crucial for the synthesis of GLA in *M. circinelloides* [323,324].

M. circinelloides are called "microbial cell factories" due to their potential to produce essential amino acids, lipids, pigments, enzymes, polyphenols, and organic acids. It is also defined as a model organism to study the biosynthetic mechanism of carotenoids, beta-carotene, and astaxanthin. *Mucor circinelloides* CBS 277.49 strain is primarily responsible for the synthesis of beta-carotene [325]. It is also used for the bioremediation process and treatment of wastewater, like the removal of polyphosphates and heavy metals. *Mucor circinelloides* SNDM1 have been previously used to remove phosphorus from wastewater as this strain can perform heterotrophic nitrification and aerobic denitrification. Both of these processes are crucial for the simultaneous removal of N and P. The removal of P by *M. circinelloides* involves its transformation into cell membranes and extracellular polymeric substances. Specifically, orthophosphate is the major intracellular P species, while polyphosphate and pyrophosphate are found extracellularly. The optimal conditions for P removal by this strain include a C/N ratio of 25–30, salinities of 0–3%, and a pH of 7.5, achieving a phosphate removal rate of 0.97 mg/L/h [326]. Besides this, it is also known for its ability to

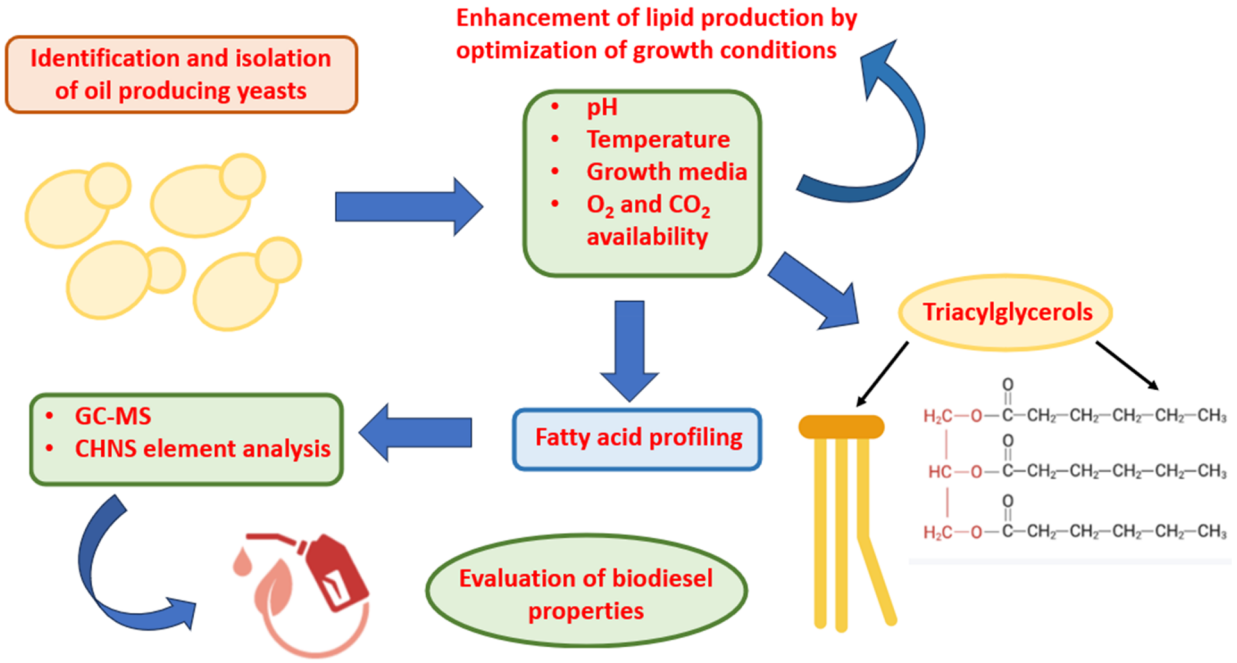

Figure 14. Identification, characterization, and production of biodiesel and oil from oleaginous fungi.

produce lipids, which are valuable for industrial applications, such as biodiesel production [327]. The fungus has been genetically engineered to increase the production of eicosatetraenoic acid (ETA), a polyunsaturated fatty acid with health benefits, demonstrating its versatility in producing high-value bioproducts [328]. *Mucor circinelloides* is also used in the production of biosurfactants, which are eco-friendly alternatives to chemical surfactants. These biosurfactants have applications in various industries, including food, agriculture, cosmetics, and pharmaceuticals [329]. The ability of *M. circinelloides* to degrade environmental pollutants, such as lambda-cyhalothrin, a pesticide, further underscores its potential as a bioremediation agent. This capability is facilitated by its rapid growth and simple growth conditions. Table 9 shows the summarized roles of fungi and their metabolites in food industries.

8. Challenges and limitations in fungal metabolite utilization

Fungal SMs have numerous applications in medicine, agriculture, dairy, and the food industry, but their practical uses face several challenges. For instance, one of the major challenges is technical hurdles in their isolation and production and the cost involved in scaling these processes. The production of these fungal SMs at larger scales is challenging due to the complex biosynthetic pathways, low yields, and instability of the product. Besides this, some of the fungi are non-culturable in the laboratory under normal conditions, while some of the SMs are produced in very small quantities or may degrade very quickly, which increases their cost. No doubt, genetic engineering and synthetic biology have played a significant role in improving production, but scaling these methods requires significant investment in infrastructure and expertise. Further investigation is required in fungal genetics, metabolic engineering, bioprocess optimization, and cost-effective production methods to overcome these issues. To use these fungal-derived SMs safely and responsibly, rigorous testing, transparent safety reporting, and regulatory engagement are required.

Besides this, some strict regulations are required to ensure the safety of fungal metabolites in food, medicine, etc., as some of the mycotoxins may be allergic and may adversely affect the individuals. Besides this, strict regulation is also needed for genetically modified fungi. Fungal metabolites struggle to compete with cheaper, more stable, and easier-to-produce synthetic compounds. Researchers, industry leaders, and legislators must work together to show fungal metabolites' environmental and economic benefits and increase production.

9. Future prospects and innovations

Due to the continuous advancement in genomics, synthetic biology, artificial intelligence (AI), and machines, there is a huge transformation in the study of fungal SMs, which makes it more attractive in biotechnology, medicine, agriculture, and other industries. Next Generation Sequencing (NGS) shows that BGCs produce important SMs, while analytical techniques like LC-MS, NMR, and high-throughput screening accelerate development. CRISPR-Cas9

Table 9. Summarized roles of fungi and their metabolites in food industries.

Fungus	Secondary metabolite	Role in food industry	References
Aspergillus niger	Citric acid	Commonly used as a preservative, acidulant, and flavor enhancer in beverages, jams, and sauces.	[330]
Aspergillus oryzae	Amylase, protease	Used in the production of fermented foods like soy sauce, sake, and miso through enzymatic breakdown of starch and proteins.	[331]
Fusarium venenatum	Mycoprotein	Used to produce Quorn, a meat substitute, by providing a high-protein, low-fat ingredient for vegetarian food products.	[332]
Geotrichum candidum	Lipases, proteases	Used in cheese ripening (e.g., Brie) to develop flavor and texture through the breakdown of fats and proteins.	[333]
Mucor circinelloides	Lipases, proteases	Used in the production of fermented dairy products, like cheeses, by breaking down fats and proteins for flavor development.	[334]
Monascus purpureus	Monacolin K, monascorubrin	Produces natural pigments used as food colorants in fermented products like red yeast rice and also lowers cholesterol in certain applications.	[335]
Penicillium camemberti	Camembertol, cyclopiazonic acid	Used in Camembert cheese ripening, contributing to flavor, aroma, and texture.	[336]
Penicillium roqueforti	Penicillic acid, roquefortine	Plays a key role in the production of blue cheeses like Roquefort, adding flavor and texture.	[337]
Rhizopus oligosporus	Protease, lipase	Key fungus in tempeh production (fermented soybeans), breaking down proteins and fats for enhanced nutritional value and digestibility.	[338]
Saccharomyces cerevisiae	Ethanol, carbon dioxide	Used in baking and alcoholic beverage production through fermentation, generating carbon dioxide for leavening and ethanol in beer and wine.	[336]
Trichoderma reesei	Cellulases, hemicellulases	Produces enzymes used in food processing, particularly for breaking down plant-based materials in the production of fruit juices and alcoholic beverages.	[339]

facilitates accurate manipulation of the genome to generate novel organic chemicals or enhance synthesis. AI and machine learning technology improve fermentation methods, forecast metabolite generation, and ease discovery by analyzing environmental DNA (eDNA). The biodegradability and nontoxicity of fungal SMs make them viable substitutes for laboratory-synthesized chemicals in a sustainable manner. Their application of eco-friendly products like biopesticides, biofuels, enzymes, and bioplastics contributes to the progress of circular economy concepts. Effective collaboration among academia, industry, and government, together with consumer education and transparent labeling, will smooth the expansion of commercialization. In conclusion, technological advances and sustainable strategies will drive the adoption of fungal SMs for a more sustainable future.

10. Conclusion

The present study emphasizes the critical importance of fungal-derived secondary metabolites in health care, agriculture, biotechnology industries, and environmental sustainability. Fungal SMs have a diverse chemical structure with potent biological activities, which represent a valuable source of novel therapeutic compounds. The advancement in genetic manipulation, for instance, CRISPR-Cas9 and heterologous expression systems, have further enabled the discovery and optimization of these fungal secondary metabolites, especially for pharmaceutical and agricultural uses. In light of the extensive production of SMs, several things are to be kept in mind before the identification of promising strains, including various epigenetic mechanisms, global regulatory elements, signal transduction pathways, and pathway-specific transcription factors involved in the production of SMs.

Still, continuous challenges persist when it comes to the enhancement of the scalability and commercial viability of SM production. These challenges could be addressed by emphasizing improving fermentation technologies and optimizing culture conditions. Due to the biodegradable and eco-friendly nature of these fungal secondary metabolites, there are the best alternatives for the chemically synthesized compounds contributing to more sustainable practices. Further investigations are required into the regulatory mechanisms of secondary metabolite production. Biotechnological innovations hold the promise of revealing the full potential of fungi as microbial cell factories.

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Ethical approval

Not applicable.

Consent to publish

All the authors have given their consent to publish this article.

Authors contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Khushbu Wadhwa, Neha Kapoor, Hardeep Kaur, Mohd. Tariq, Sazada Siddiqui, Virendra Kumar Yadav, and Pankaj Kumar. The first draft of the manuscript was written by Khushbu Wadhwa, Neha Kapoor, and Hardeep Kaur. The review and editing, software, and proofreading were done by Eman A. Abu-Seer and Saad Alghamdi, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Disclosure statement

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Data availability statement

No datasets were generated for this manuscript.

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