REVIEW ARTICLE Agricultural Reviews, Volume 47 Issue 3: 355-364 (June 2026)

Melanin: Properties, Biosynthesis and its Role as Virulence in Fungi: A Review

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10.18805/ag.R-2766

ABSTRACT

Melanin in fungi has been postulated to be involved in a wide range of virulence-associated properties which include interactions with the hosts, oxidative stresses, UV light and hydrolytic enzymes, resistance to antifungal agents, iron-binding activities and even the harnessing of ionizing radiation in contaminated soils. Melanin is found to be a secondary metabolite as it is produced after the cessation of active growth of fungus. They play an important role in fungal pathogenesis. In *Colletotrichum lagenarium* and *Magnaporthe grisea,* melanins are essential for infectivity, as they allow enormous pressure to build in appressoria, enabling the fungus to penetrate plant leaves. Fungal melanin diversity was reported to occur through two biosynthetic pathways. Melanized fungi more effectively prevent diffusion of glycerol which retains a higher turgor pressure necessary for the degradation of the cuticle. A notable example of this phenomenon is the fungus *M. grisea* which utilizes melanin to invade rice plants and result in rice blast disease.

Key words: DHN biosynthesis, DOPA pathway, Fungal infections, GDHB melanin, Inhibitors, Melanin.

Colours have been a vital and variable constituent for living organisms due to the presence of various secondary metabolites called pigments. Pigments are the material that changes the colour of reflected or transmitted light as the result of wavelength-selective absorption. Microbes produce multiple types of biological pigments in nature, ranging from monomeric (*i.e.* carotenoids, luciferin, flavonoids and heme/porphyrin-based, such as chlorophylls, bilirubin, haemoglobin, haemocyanin) to polymeric (*i.e.* melanins, tannins and humic substances). Pigments serve various biological functions like camouflage or makeup to fundamental roles in life maintenance including harnessing solar energy for metabolic use and protection against radiation damage. In order to survive extremes of pH, temperature, salinity, radioactivity and host defenses, microorganisms have been found to develop unique protective mechanisms (Cordero, 2017). The term melanin originates from "*melanos"* a Greek word for black. Melanin is a class of compounds found in plants, animals, fungi, and protists. The exact structures of melanins are unidentified. In general, melanins are hydrophobic pigment biopolymers formed by oxidative polymerization of phenolic or indolic compounds. Melanin is produced in the environment by a process called melanization which is important for adaptation to unfavourable life conditions (Singh *et al*., 2013). Adaptation to extreme conditions especially towards two or more extreme factors is usually seen as successful in fungi. Melanin in fungi has been postulated to be involved in a wide range of virulence-associated properties, including interactions with hosts, oxidative stresses, UV light, and hydrolytic enzymes; resistance to antifungal agents; iron-binding activities; and even the harnessing of ionizing radiation in contaminated soils (Gessler *et al*., 2014; Casadevall *et al*., 2012).

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How to cite this article: Valarmathi, P. (2026). Melanin: Properties, Biosynthesis and its Role as Virulence in Fungi: A Review. *Agricultural Reviews*. **47(3):** 355-364. doi: 10.18805/ag.R-2766.

Submitted: 14-10-2024 **Accepted:** 10-03-2025 **Online:** 12-05-2025

Definition

Melanins are dark brown/ black secondary metabolites made up of complex heterogeneous polymers of phenolic and/or indolic monomers. These are conjugated polymers of ortho dihydroxy phenols. Derived from the precursor molecule 1, 8-dihydroxy naphthalene (DHN) and are known as DHN-Melanins (Bell and Wheeler, 1986).

Properties of melanin

- These are not essential for growth and development. They enhance the survival and competitive abilities of speciesin certain environments. Provide defence against environmental stress.
- They play an important role in fungal pathogenesis. In Phytopathogens-*Colletotrichum lagenarium* and *Magnaporthe grisea* melanins are essential for nfectivity, as they allow enormous pressure to build in appressoria that enable the fungus to penetrate plant leaves.
- They have been related to protection from desiccation, stress resistance, virulence and energy transduction.

Occurrence/abundance of melanins

Melanin is predominantly restricted to the basal layer (or Stratum basale) of the human epidermis, where it is found

in both melanocytes (MCs), the cells that make melanin, and in nearby keratinocytes (KCs), the cells of the epidermis that accept melanin. Melanin can be found in the inner or outer layers of the cell wall depending on the fungal species. In pathogenic fungi, melanins are often reported to be associated with or "in" the cell wall. It is the predominant pigment used in animals and is found in two forms- eumelanin produces black to grey coloration and pheomelanin produces yellow to red coloration. Melanin molecules are deposited in integumentary tissue including skin, fur, hair, feathers and cuticle.

Animal and plant melanins

Eumelanins are predominant forms found in animals and microorganisms and occur in some fungi; pheomelanins are specific of higher animals, mammals, or birds. Both of them are derivatives of tyrosine, but pheomelanins consist of sulfur-containing monomeric units, mostly benzothiazine and benzothiazol, instead of indole units in eumelanins. Plant and fungal melanin, devoid of nitrogen is generically named as allomelanin (other melanins). It is the most heterogeneous group; its precursors are varied. Fungal melanin can be formed from gamma-glutaminyl-3,4-dihydroxybenzene, catechol and 1,8-dihydroxynaphthalene, while catechol, caffeic, chlorogenic, protocatechuic and gallic acids are considered to be the possible precursors in plants (Glagoleva *et al.,* 2020). Eumelanin is found in many animals and has various effects. Bird feathers, for example, are a mixture of eumelanin and pheomelanin. Their levels vary with the species of bird. Interestingly, the concentrations of eumelanin and pheomelanin in female feathers are positively correlated with male feathers. Eumelanin is also found in marine cephalopods, such as cuttlefish, whose ink contains a special kind of melanin called squid ink melanin. It is formed by irregular isomers of indole and carboxypyrrole units with some related proteins. During the production of melanin, hydrogen peroxide and free radicals are produced during its polymerization. The generated free radicals lead to the appearance of melanin units (such as carboxypyrrole) as a result of the rupture of the hexagon ring of the indole unit. Benzothiazine- or benzothiazole-like pigment is mainly yellowish or reddish and is present in high levels in poultry feathers, human red hair, insects and amphibians, as well as in reptiles, but in very rare amounts.

Melanins provide additional mechanical strength to seed shells, protecting them from damage. Moreover, melanin provides resistance to insects and pests due to its toxicity. In sunflower, seeds with black seed coats are less damaged by mole larvae than white seeds. As melanins are strong antioxidants, they can confer more vigor to seeds that accumulate them and can protect seeds under stress. There are some examples to support this hypothesis. In watermelon, the brown seeds were more vigorous than the light-colored seeds; they had higher seed weight, germination and emergence percentages and seedling fresh and dry weight than light-colored seeds (Mavi, 2010). In *Brassica* species, yellow seeds with transparent seed coatings have thinner hulls and less fiber than varieties with dark, thicker and more lignified seeds. Melanin synthesis in plants is associated with the enzymatic browning reactions that occur in damaged tissues by polyphenol oxidases (PPOs), which belong to a family of Cu-containing oxidoreductases that are able to act on phenols in the presence of oxygen.

Difference between animal and fungal melanin.

Animal melanin	Fungal melanin
➢ Synthesized from Tyrosine	➢ Synthesized from acetate
➢ Key enzyme-tyrosinase	➢ Key enzyme-pentaketide synthase
➢ Synthesized only in specialized dendritic	➢ Synthesized and occur either in cell walls or as extra-cellular polymers in and around fungal cells.
➢ Cells called melanocytes and melanophores.	
➢ Melanin granules are dispersed in the cytoplasm	

Types of melanins

Melanins are classified based on the colour and the constituents from which they have been derived (Fig 1 and Table 1). Depending on the precursors, the resulting products of polymerization are the brown-black eumelanin, the yellow-red pheomelanin, and a heterogeneous group of allomelanins, including piomelanins and DHN-melanin formed via the polyketide pathway, a very common in fungi.

Eumelanins

Eumelanins are dark brown to black pigments with 6-9 % nitrogen and 0-1% sulfur. They are the oxidation products

Table 1: Types of melanins, its sources and precursor.

Type of melanin	Producing sources	Melanin precursor
Eumelanin (DOPA-melanin)	Animals, bacteria, fungi	Tyrosine or L-Dopa
Pheomelanin	Animals	5-S-cys-Dopa
Neuromelanin	Human (brain)	Dopamine and 5-S-cys-dopamine
Neuromelanin	Plants	Catechol
Allomelanin (DHN-melanin)	Fungi, bacteria	1,8-dihydroxynaphthalene (DHN)
Pyomelanin	Fungi, bacteria	Homogentisic acid

of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA). The predominant color of eumelanin is from black brown, which is found in human hair and skin. It is also produced by some bacteria, fungi, and myxomycetes. Some myxomycetes can also produce melanin, and as a separate taxon of protists, myxomycetes can become dormant forms of plasmodia under adverse environmental conditions. Myxomycetes are able to produce melanin in spores and melanin content is greater in dark spores than in bright spores.

Pheomelanins

Pheomelanins are reddish-brown pigments with 8-11% nitrogen and 9-12% sulfur, composed of benzothiazine monomer units. Allomelanins show a heterogeneous group of pigments derived from metabolites of homogentisic or p-hydroxy phenylpyruvic acid (piomelanins), γ-glutamyl-4-hydroxybenzene and catechols (Nappi and Ottaviani, 2000; Espin *et al.,* 1999; Bell and Wheeler, 1986). Melanins formed from DHN also belong to allomelanins. They are very common in fungi and typically do not contain nitrogen.

Neuromelanins

As a mixture of eumelanin and pheomelanin, neuromelanin is the only melanin pigment that is not formed in melanocytes but in catecholaminergic neurons of the substantia nigra. It has been shown that neuromelanin contains benzothiazine and indole units, with cysteine in its pheomelanin core and eumelanin on its surface. Neuromelanin is a dark insoluble pigment produced in different areas of catecholaminergic neurons in the brain. Nigrasomes should contain pheomelanin in the core and eumelanin on the surface (Bush *et al.,* 2006).

Allomelanin

Allomelanins are heterogeneous pigments with a nitrogen-free heterogeneous polymer class found in many fungi and plants. They come from many sources, including

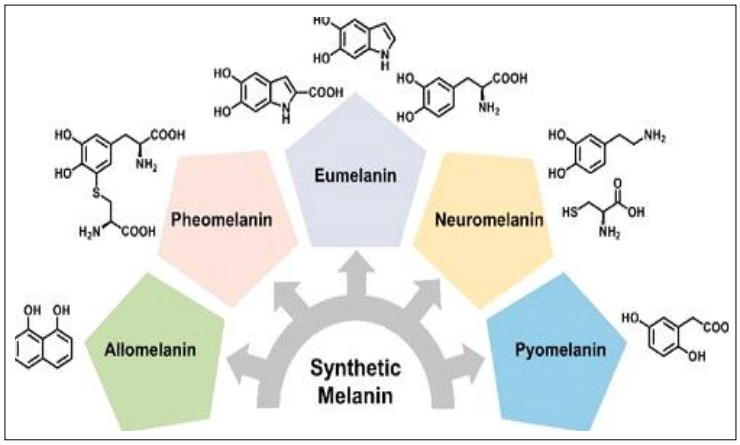

Fig 1: Types of melanins (Cao *et al.,* 2021).

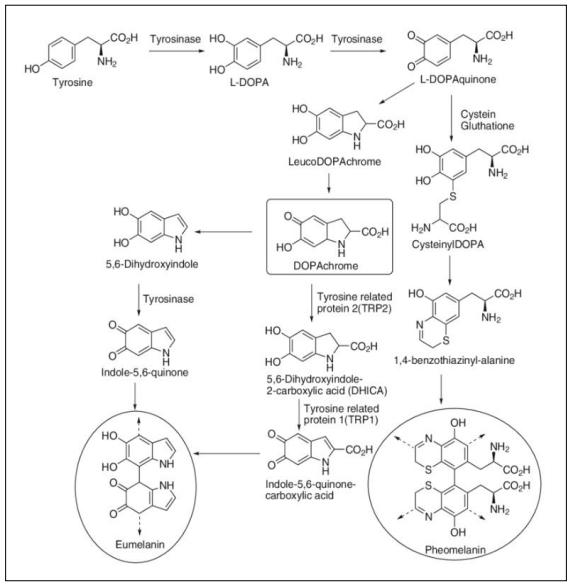

Fig 2: Melanin biosynthesis pathway in fungi.

dihydrofolic acid, hyperic acid, catechol, *etc.* Allomelanin is a group of heterogeneous polymers emerging from the oxidation or polymerization of di-DHN (1,8-dihydroxy naphthalene) or THN (1,3,6,8-tetrahydroxy naphthalene) to produce DHN-melanin or allomelanin in various colors (Bush *et al.,* 2006).

Pyomelanin

Unlike eumelanin, pheomelanin does not have its biosynthetic pathway but is associated with the activation of the L-tyrosine/L-phenylalanine degradation pathway. It is a water-soluble pigment that is produced during the accumulation and polymerization of homogentisic acid as a by-product of the tyrosine catabolic pathway. In the tyrosine catabolic pathway, the deamination of tyrosine generates p-hydroxyphenylpyruvate, which is oxidized to homogentisic acid by further oxidation and decarboxylation. The final formation of pheomelanin is achieved through cyclic rearrangement and successive polymerization of homogentisic acid (Bush *et al.,* 2006).

Melanin biosynthesis in fungi

Biosysnthesis of fungal melanin was reported to occur through two biosynthetic pathways: the acetatemalonate pathway and shikimic acid one. Polymerisation of phenolic compounds that are catalysed by copper-based enzymes results in the formation of high molecular weight melanins. Copper is important for melanin biosynthesis in both the DHN and L-dihydroxyphenylalanine (DOPA) pathways (Fig 2) (Chang, 2009).

The DHN-melanin biosynthesis

Fungal melanins are one of the important secondary metabolites, in which the majority are derived from the precursor molecule 1,8- DHN and are known as DHN-melanins. The production is primarily found in Ascomycetes and related Deuteromycetes and furnishes DHN by polyketide pathway. Recognized human pathogens which form melanin precursors by the polyketide pathway include *Aspergillus nidulans, A. niger, Alternaria alternata, Cladosporium carionii, Exophiala jeanselmei, Fonsecaea compacta, F. pedrosoi, Hendersonula toruloidii, Phaeoannellomyces wernickii, Phialophora richardsiae, P. verrucosa, Wangiella dermatitidis* and *Xylohypha bantiana* (Alspaugh *et al*., 1998). A five-carbon acetate (Malonyl-CoA) serves as the starter and extender unit for the polyketide synthase (PKS1) catalyzing the first step in the biosynthesis pathway. Malonyl CoA is first converted into an intermediate *i.e.* 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN) by polyketide synthase (PKS). Following this, the formed intermediate (1,3,6,8- THN) is reduced by a specific reductase enzyme to scytalone. It was discovered that a specific reductase inhibitor, tricyclazole, produced the same defect as a mutation in the reductase gene, namely the accumulation of flaviolin, a shunt product of 1,3,6,8- THN. Scytalone is dehydrated enzymatically to 1,3,8-trihydoxynaphthalene, which is further reduced, by a second reductase enzyme to vermelone which can be inhibited also by tricyclazole (Alspaugh *et al*., 1998). Subsequent steps are thought to involve dimerization of the 1,8-DHN molecules, followed by polymerization, possibly catalyzed by laccases, phenol oxidases, peroxidases and catalases (Sugareva *et al*., 2006; Zheng *et al*., 2002). This is a general model for DHN-melanin biosynthesis, but the pathway (and the resulting colour) may vary in different fungi. Interestingly, various studies reported that several by-products of the fungal DHN-melanin pathway have been shown to have antibacterial or immunosuppressive properties. In fungi, melanin biosynthesis could begin through metabolites of the shikimic acid pathway.

L-3,4-Dihydroxyphenylalanine-melanin biosynthesis (DOPA pathway)

DOPA melanin-synthesizing fungi include many model organisms such as *Neurospora crassa, Podospora anserina, Aspergillus nidulans, Aspergillus oryzae* and also pathogenic fungi such as *Cryptococcus neoformans*. Precursors like tyrosine or phenylalanine oxidized by tyrosinase or laccase into DOPA result in the formation of eumelanins. The formation of an intermediate product DOPA from tyrosine is catalysed by the tyrosinase enzyme which is found in many fungi like *Agaricus bisporus, N. crassa, Tuber melanosporum, T. manatum* (Singh *et al*., 2013; Butler and Day, 1998). The expression of this enzyme is closely related to the developmental stages and pathogenesis of fungi (Zarivi *et al*., 2011). In *Lentinula edodes* and *C. neoformans* DOPA is converted into melanin with the participation of laccases (Nagai *et al*., 2003). The second DOPA oxidation step is manifested in DOPA quinone formation, followed by the cyclization and building up of DHI or DHICA with their following oxidation to indole-5,6-quinone or indole-5,6-quinone carboxylic acid. Amino acids with sulfur compounds like cysteine or glutathione which liberate systems through the action of a glutamyltranspeptidase are involved in the pheomelanin synthesis pathway. In the presence of cystines, DOPA-quinones connect with cystines to form 5-S-cysteinyl-DOPA and 2-S-cysteinyl-DOPA which give benzothiazin intermediates that polymerize to produce pheomelanins-brown, red, or yellow pigments (Almeida-Paes *et al*., 2012; Kobayashi *et al*., 1995). An alternate DOPA pathway of melanin biosynthesis in fungi includes tyrosine transaminase providing the formation of 4-hydroxyphenylpyruvate, which is further converted into homogentisic acid by dioxygenase and is then spontaneously oxidized to benzoquinone acetate and polymerized resulting in the formation of soluble brown piomelanins. Soluble piomelanins formed from tyrosine via hydroxyphenylpyruvate and homogentisic acid were found in *Aspergillus fumigatus, Aspergillus kawachii, Madurella mycetomatis* and *Yarrowia lipolytica* (Almeida-Paes *et al*., 2012).

Glutaminyl-4-hydroxybenzene melanin (GDHB melanin)

Good evidence for the presence of melanin is the basidiospore wall melanin of *Agaricus bisporus,* generated from the precursor glutaminyl-4-hydroxybenzene (GHB) which is synthesized via the shikimate pathway. GHB is converted to glutaminyl-3, 4-dihydroxybenzene (GDHB) which on further oxidization by catalyzing with peroxidase or phenolase forms γ-glutaminyl-3, 4-benzoquinone and these are polymerized later (Nappi and Ottaviani, 2000). It is the immediate precursor to the spore wall melanin. GDHB is found only in reproductive hyphae that form melanized spores. The polymerized products of γ-glutaminyl-3, 4-benzoquinone, benzoquinone acetate, and 1,8-DHN form a heterogeneous group of allomelanins. Studies revealed that the majority of the basidiomycetous mushrooms contain GHB and GDHB which indirectly leads to a conclusion that DHN melanins may be produced by ascomycetes fungi, while GDHB melanins production may be restricted to the basidiomycetous fungi.

Melanin localisation in fungi

Melanin localization studies have shown that these pigments in fungi may be detected in the cell wall or secreted into the environment (Butler *et al*., 2005). A mix of cross-linked fibres (the polysaccharides glucan and chitin) and matrix components (primarily proteins and mannans) are incorporated into the fungal cell wall. The fungal cell wall contains mannoproteins in the outer layer and the inner layer constitutes mainly polysaccharides (β-glucans and chitin) and small amounts of proteins (Osherov and Yarden, 2010). The localization of the melanin may also vary between the species, external to the wall found in *P. brasiliensis* (Taborda *et al*., 2008). Dark melanin granules were found in the fibrillar matrix on the surface of the cell wall in *Aureobasidium pullulans, Verticillium dahliae* and *Phomopsis* spp (Caesar-Tonthat *et al*., 1995).

A combination of SEM and TEM microscopic studies shows that melanin has an overall granular structure. The granules are localized to the cell wall where they are likely cross-linked to polysaccharides in various fungi (Chatterjee *et al*., 2015; Casadevall *et al*., 2012). According to electron microscopy of the soil fungus *Gaeumannomyces graminis*, the melanin layer composes almost half of the thickness of the cell wall and is located between the cell wall and the inner chitinous layer (Caesar-Tonthat *et al*., 1995). In *Sclerotinia sclerotiorum*, melanins form a solid protective layer on the outer layer of the sclerotia (Butler and Gardiner, 2009). In the multicellular conidia *Alteranaria Alternata* (Carzaniga *et al*., 2002), melanin was localized in the outer layer of the cell wall. Hence the localization and participation in cell wall porosity make melanin an excellent defense system against unfavourable environments and antifungal drugs, as well as a factor in host-parasite interactions. Functional properties of fungal melanin includes biosorbent, antibacterial,antioxidant and anti-radiation, photoprotection (Fig 3).

Characterization of melanin

With concerted efforts over time, it became possible to elucidate the proper structure of melanin using various techniques, such as UV and visible spectroscopy, elemental analyses, physico-chemical tests, FTIR (fourier transform infra-red spectroscopy), EPR (electron paramagnetic resonance) and NMR (Nuclear magnetic resonance) (Fig 4). At a primary stage, basic determinations of solubility and UV spectra can also provide relevant information in a short time. Due to the heterogeneous nature of melanin, it lacks a well-defined structure. Therefore, meticulous characterization techniques are taken into account so as to ascertain the structure of melanin (Suthar *et al.,* 2023).

Extraction, purification and quantification of melanin from *Bipolaris oryzae*

Melanin is a dark-pigmented polymer that protects organisms against environmental stress and its production is also widespread in the fungal kingdom which accumulates in fungal cell wall. The expression of three melanin biosynthesis genes involved in the melanin biosynthesis, polyketide synthase gene (PKS1)19, scytalone dehydratase gene (SCD1)20 and 1,3,8-THN reductase gene (THR1) 21 was specifically up-regulated by near-UV (NUV: 300-400 nm) radiation in *B. oryzae*. The information on melanin content and pathogenicity of *B. oryzae* in rice host has not been studied earlier. Extraction and estimation of melanin from the hyphae was performed following the methodology given as follows. Mycelium was scraped from 5 days old colonies of each isolates boiled for 5 min in 5 ml distilled water and centrifuged (5,000 g, 5 min). The mycelial pellet was then washed, centrifuged again and the pigment was extracted by autoclaving the pellet with 3 ml (1 M) NaOH for 20 min at 120°C. This was followed by acidification (pH 2) of the alkaline pigment extract with concentrated HCl to precipitate the melanin.

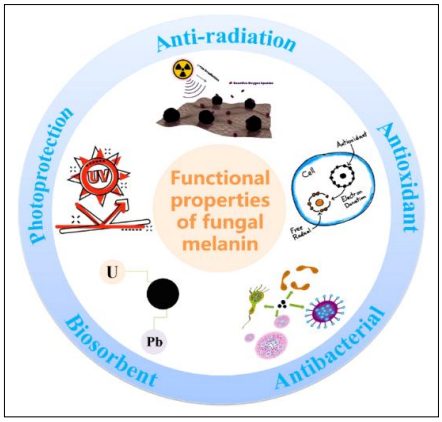

Fig 3: Functional properties of fungal melanin (Liu *et al*., 2022)

The precipitate was washed thrice in distilled water and dried overnight at 20°C in a dehumidified condition for further analysis. Melanin content was measured spectrophotometrically at 405 nm and melanin content (µg/g of mycelium) was determined using a standard curve generated from pure melanin. The most virulent isolate of *B. oryzae* BO 1 was shown to have high melanin content than the other isolates. There is positive correlation (r= 0.79) observed between pathogenicity and melanin content among the *B. oryzae* isolates. In earlier studies a significant positive correlation (r= 0.7) was obtained between sporulation and melanin content in *B. sorokiniana* (Kartar *et al.,* 2016). Therefore, in future the expression of melanin biosynthetic genes involved in virulence of the pathogen has to be studied in details.

Role of fungal melanin in plant infections

The melanin in melanized fungi can also play a surprisingly crucial role beyond human and insect hosts. Black fungal pathogens have a significant impact on agriculture globally. One example is the species *Colletotrichum.* This species predominantly causes anthracnose disease, red rot, crown rot, and brown blotch. The fungi are so expansive that they affect: papaya, citrus, strawberry, tomato, corn, alfalfa, pepper, legumes, radish, coffee, and sorghum. More specifically, melanized fungi use melanin to create and maintain high turgor pressures in the appressorium while inserting themselves into the plant hosts, absorbing essential minerals which function as a reservoir for the fungi and preventing loss of glucose (Nosanchuk and Casadevall, 2003). The melanin produced by fungal plant pathogens plays a significant role in the colonization of the plant host. To colonize a plant host, fungi produce appressoria, or tiny hyphal cell formations containing glycerol, which help create enough turgor pressure to penetrate the epidermal cells of plants. Specifically, melanized appressoria are comparatively advantageous to non-melanized appressoria in terms of generating and maintaining sufficient turgor pressure to invade the plant. Melanized fungi more effectively prevent diffusion of glycerol

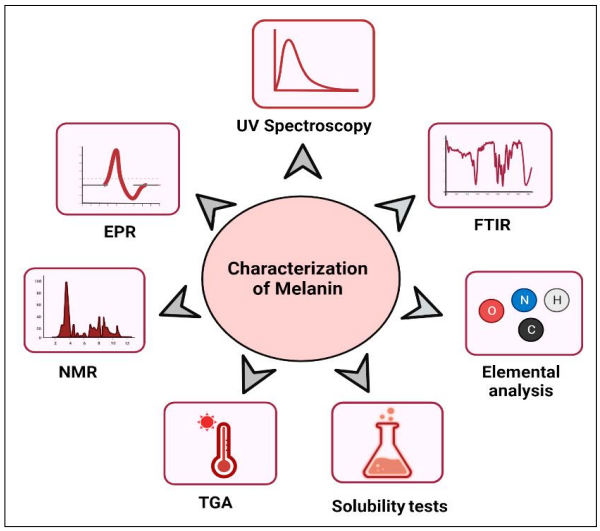

Fig 4: Various methods for the characterization of melanin (Suthar *et al.,* 2023).

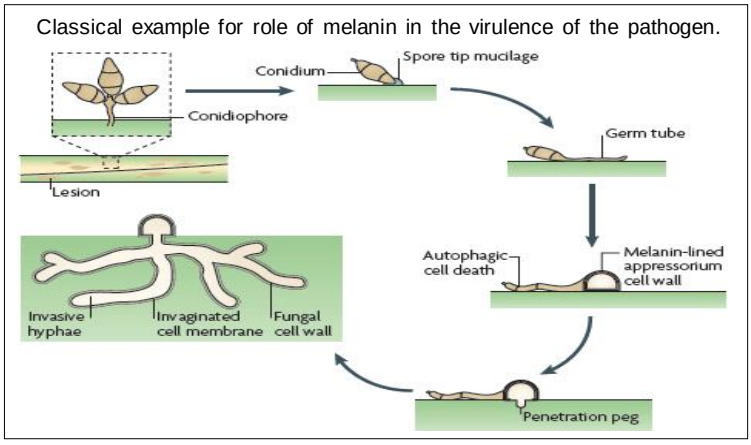

Fig 5: Appressorial penetration mechanism of *Magnaporthe grisea* into rice epidermis (Wilson and Talbot, 2009).

which retains a higher turgor pressure necessary for the degradation of the cuticle. A notable example of this phenomenon is the fungus *Magnaporthe grisea* which utilizes melanin to invade rice plants and result in rice blast disease (Howard and Valent, 1996). When the same *M. grisea* is treated with a tricyclazole, a reagent that prevents the synthesis of melanin, or an albino mutant of *M. grisea* is used, the appressoria are unable to generate sufficient turgor pressure (Howard and Valent, 1996). Appressorial penetration mechanism of Magnaporthe grisea into rice epidermis has been provided in Fig 5. The role of melanin in melanized fungi is not limited to just host-invasion processes; it can also play a defensive role. Fig 6 and Fig 7 explained the SEM and TEM of Magnaporthe grisea. Melanin can aid the survival of melanized fungi even while in a dormant state (Butler *et al.,* 2001). Fungi which produce melanized sclerotia, a bundle of hyphae, are far more resistant to chemical attacks; this is incredibly important for resistance against fungicides as well. For fungi that do not produce sclerotia, melanin still plays a role in protecting the fungi. This is evident, for example, in mutant versions of fungi *G. Graminis* which are more susceptible to ultraviolet radiation, lytic enzymes and some antimicrobial agents. Melanotic fungi pose clear offensive and defensive advantages over their non-melanized counterparts. More specifically, melanized fungi are more effectively able to invade plants, maintain nutrients and protect themselves against an array of chemical, radioactive, and other physical threats. A bacterial strain *B.amyloliquefaciens* B6 which was isolated from *O. officinalis* showed inhibitiory activity against the rice sheath blight pathogen, *R. solani*. Antifungal secondary metabolites from endophytic *B. amyloliquefaciens* B6 was responsible for the suppression of *R. solani* under *in vitro* condition (Sirivella Naveena *et al.,* 2025).

Melanin inhibitors

Fungal melanin is synthesized through the polyketide pathway which includes the fusion of five isoprenyl units, two steps of reduction, two steps of dehydration, and polymerization of 1,8-dihydroxynaphthalene. After resolving the melanin biosynthesis pathways in *Magnaporthe oryzae, Verticillium*, *Colletotrichum*, *Cochliobolus* and *Alternaria* (Bell and Wheeler, 1986) importance of melanin biosynthesis inhibitors development has been increased for the control of these diseases especially rice blast as the other pathogens such as *Cochliobolus heterostrophus* and *Alternaria alternata* require no melanin in the infectious process into the host plants. The biosynthesis of melanin

Fig 6: a) Rice blast symptom on seedlings, b) Neck blast, c) Large rice blast lesions on mature rice plant, d) SEM of *M. grisea* conidia, e) SEM of a dome-shaped appressorium of *M. grisea* on the rice leaf surface (Wilson and Talbot, 2009).

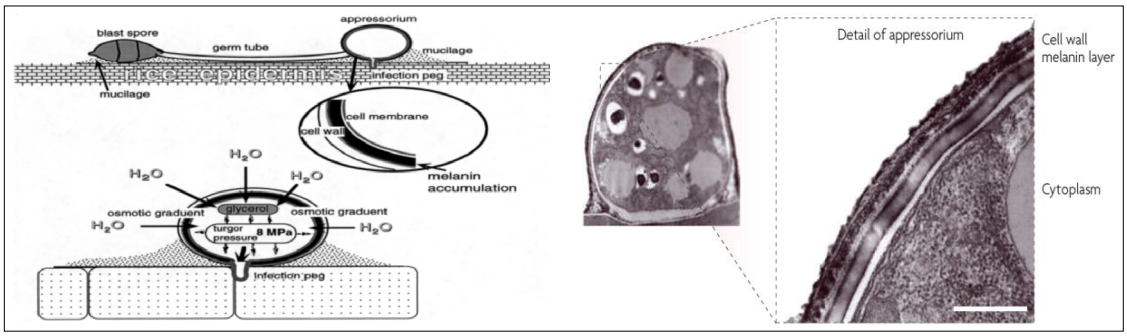

Fig 7: The *M. grisea* appressorium cell wall. TEM of a transverse section of an appressorium (Wilson and Talbot, 2009).

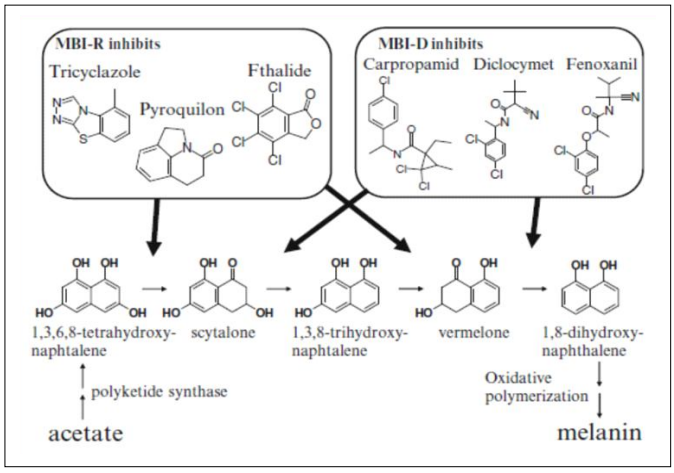

Fig 8: Mode of action of melanin biosynthesis inhibitors (MBIs).

is essentially required in *M. oryzae* and *C. lagenarium* (Bell and Wheeler 1986). The melanin biosynthesis inhibitors based on the mode of action are divided into two groups, they are MBI- Dehydratases (MBI-D) and MBI-Reductases (MBI-R). Scytalone dehydratase (SH) inhibitors, including melanin biosynthesis dehydratase inhibitors (MBI-Ds), form one group of MBIs. Different compounds like carpropamid, diclocymet, and fenoxanil, are included in the MBI-D group as they block the dehydration reactions from scytalone to 1,3,8-trihydroxynaphthalene and from vermelone to 1,8-dihydroxynaphthalene (Fig 8).

Carpropamid is a long-acting agent mainly used for nursery-box treatment and has contributed to saving labour and lessening application frequency since 1998 in Japan for controlling rice blast showing a systemic action (Kurahashi *et al*., 1997). Later diclocymet and fenoxanil, were launched in 2000 and 2001, respectively. In 2001, however, resistance to carpropamid was reported in the southern part of Japan, where MBI-Ds have been used since 1998 (Yamaguchi, 2004). Isolates of *M. oryzae* from the southern part of Japan showed decreased sensitivity to carpropamid *in-vivo* and also been shown that these isolates displayed cross-resistance to the fungicides: diclocymet and fenoxanil, which are also categorized in the MBI-D class (Sawada *et al*., 2004). Till now no resistant strains to MBI-Reductase inhibitors have been reported, though it has been used for over 40 years.

List of melanin inhibitors

1β-hydroxy arbusculin A, 4,5,6,7-tetrachloro-2-benzofuran-1(3*H*)-one, N^2-([biphenyl]-4-ylsulfonyl)-*N*-hydroxy-N^2-isopropoxy-D-valinamide, arbusculin A, arpropamid, diclocymet, fenoxanil, fucoidan, geranic acid, lespeflorin, monobenzone, pyroquilon and tricyclazole.

At increased concentrations (10% and 15%), improvement of leaf color (becomes yellow due to blight disease by *F. oxysporum*) root length (root rot by *R. solani*) and stem length (becomes brown or color rot by *S. rolfsi*) because the % rate of active fungal spore is induced. Similarly, higher concentrations of extracts containing phenolics and alkaloids might have higher absorption of minerals and water and their translocation from roots to other plant parts (Sahrawat *et al.,* 2024). The production of secondary metabolites enables plants to change insect physiology and behavior which results in toxicity to insects or development of non-preference to the host (Mansura *et al.,* 2021).

Future prospects

Melanin's role is not only restricted to pathogenesis but also other roles like mechanical protection, energy harvesting, anti desiccating etc. Research on the synthesis of melanin biosynthesis inhibitors may help to control the pathogens that use melanin as a component for entry into the host. Molecular studies will help in identifying the melanin producing genes of the pathogen which on further help to develop the resistant varieties. Furthermore, the accumulated knowledge on the biochemistry and genetic engineering of melanin in various organisms can contribute to the direct manipulation and enhancement of melanin production. With this perspective, melanin can be used beyond basic research and encourage more researchers from industry to deploy bio-inspired melanin-based materials for biomedical, environmental and technological applications.

CONCLUSION

Melanins are dark brown/black secondary metabolites made up of complex heterogeneous polymers of phenolic and/or indolic monomers. These are conjugated polymers of ortho dihydroxy phenols. Derived from the precursor molecule 1, 8-dihydroxy naphthalene (DHN) and are known as DHN-Melanins. They play an important role in fungal pathogenesis. In Phytopathogens - Colletotrichum lagenarium and Magnaporthe grisea melanins are essential for infectivity, as they allow enormous pressure to build in appressoria that enable the fungus to penetrate plant leaves.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the concept of the study, decision to publish or preparation of the manuscript.

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