Epicoccum nigrum Link as a Potential Biocontrol Agent Against Selected Dermatophytes

Agata Piecuch*, Rafał Ogórek, Mariusz Dyląg, Magdalena Cal, Katarzyna Przywara

Department of Mycology and Genetics, Institute of Genetics and Microbiology, University of Wrocław, Poland

*To whom correspondence should be addressed. Email: agata.piecuch@uwr.edu.pl

Abstract

Epicoccum nigrum Link is well known for producing biologically-active substances with activities against prokaryotic and eukaryotic cells. The major goal of this study was to assess E. nigrum as a potential in vitro agent against selected species of dermatophytes. The effects of the types of media used in this study on the interactions between the microscopic fungi were also examined. Epicoccum nigrum's bioactive metabolites exhibited a strong growth inhibitory effect against the dermatophytes, suggesting its potential as a biocontrol agent. Notably, the strength of these interactions was dependent on the type of the medium. These secondary metabolites are not toxic against the higher eukaryotic organisms, which was further demonstrated by using the Galleria mellonella model.

Keywords

secondary metabolites; fungi; endophyte; toxicity

1. Introduction

Epicoccum nigrum Link (syn. E. purpurascens Ehrenb. ex Schlecht) is an endophytic fungal species, which is widely distributed as it is found on plant surfaces and in water, soil, and air. This species is particularly known for producing a variety of biologically-active substances. Epicoccum nigrum isolated from the marine environment produces extracellular polysaccharides with free radical scavenging activity and is potentially useful in the prevention of oxidative damage in higher organisms (Sun et al., 2011). Somjaipeng et al. (2016) showed that E. nigrum could also produce taxol, which is a diterpenoid anticancer drug, and is induced by the elicitors, like water activity or pH. Colored secondary metabolites, such as prodigiosins, which are also excreted by E. nigrum, may have a potential role as antimicrobial or antitumor compounds (Perveen et al., 2017). Epicoccum nigrum extract, which was isolated from the Ferula umbil leaves, was also found to contain prodiginine and was shown to exhibit strong antimicrobial activity against the microscopic fungi and bacteria (e.g., Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Candida albicans). It was also shown to exhibit anticancer activities against melanoma cell lines (Perveen et al., 2017). Epicoccum nigrum extraxt, which was isolated from the Ferula umbil leaves, was also found to contain prodiginine and was shown to exhibit strong antimicrobial activity against the microscopic fungi and bacteria (e.g., Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Candida albicans). It was also shown to exhibit anticancer activities against melanoma cell lines (Perveen et al., 2017). This fungus, when isolated from the cambium of Phellodendron amurense, has also been used for the extracellular synthesis of silver nanoparticles with a wide variety of biological activities (Qian et al., 2013). Epicorazines A and B, isolated from E. nigrum, exhibited antibacterial activity. Cultured E. nigrum hyphae were also shown to excrete several dyes, including β- and γ-carotene, rhodoxantin, and epicocconone, in the medium (Baute et al., 1978).

The variety of biologically-active secondary metabolites produced by E. nigrum makes it a potential organism for the biocontrol of phytopathogens. Although there is one report describing the pathogenic interaction of E. nigrum with Lotus
corniculatus (Colavolpe et al., 2018), in general, this fungus is considered to be a facultative saprotroph, exhibiting an important role in plant protection against pathogens (de Cal et al., 2009). It has previously been shown that *E. nigrum* isolated from sugarcane inhibits several phytopathogens, such as *Cyanophora paradoxa* and *Fusarium verticilloides*, and is involved in enhancing the root growth (Fávaro et al., 2012).

Although there are other reports describing the antimicrobial action of *E. nigrum* metabolites against yeast-like fungal human pathogens, the data on their effects against other classes of mycoses-causing fungi, such as dermatophytes, are rather limited. Only one example of growth inhibition of *Trichophyton mentagrophytes* has been described previously (Mallea et al., 1991). Dermatophytes are a cause of communicative diseases that are acquired from infected animals and humans. The clinical manifestations of the infections that are caused by dermatophytes include pedis and tinea capitis. The most common etiological agents causing dermatophytoses are fungal anamorphs, such as *Trichophyton* sp. and *Paraphyton* sp. (Weitzman & Summerbell, 1995).

The genus *Trichophyton* causes the infections among farm animals, mainly in calves and horses, but also in rabbits, sheep, rats, monkeys, cats, and dogs. Human infections might occur after coming in contact with an infected animal. People with impaired immunological system are particularly vulnerable to these pathogens. In contrast, the genus *Paraphyton* is comprised of anthropophilic, zoophilic, and geophilic species. Among the latter, there are fungi that cause diseases in humans but are not yet reported as pathogenic fungal strains (Weitzman & Summerbell, 1995).

It is thus important to search for new antagonists and/or biologically active substances against the dermatophytes. Since higher eukaryotes are the potential hosts of these dermatophytes (Achterman et al., 2011), it is crucial that the biological agents are not toxic against them, and thus in vitro and in vivo toxicity assays should be performed, preferably on mammals, prior to their application (Jorjão et al., 2018). Since the tests on mammals might raise several ethical issues, therefore researchers should develop other eukaryotic models, such as insects for the same. Insect systems have now been extensively used to assess the virulence of fungal pathogens and for in vivo drug toxicity assays due to their low cost, easy culture, and lack of ethical restrictions. Their innate immune system shares many similarities with that of mammals. Among many different insect models, the greater wax moth, *Galleria mellonella* has been frequently used by the scientific community (Kavanagh & Sheehan, 2018).

The major goal of our study was to assess the potential of *Epicoccum nigrum* as a biocontrol agent against dermatophytes by determining whether it shows in vitro biotic interactions with selected species of dermatophytes, as well as by assessing the effects of its secondary metabolites on the survival and growth of *Galleria mellonella* larvae.

2. Material and Methods

The in vitro antagonism between *E. nigrum* and dermatophytes were studied using the biotic series method described by Manika and Manika (1992) and Ogórek and Pląskowska (2011) on PDA (potato dextrose agar; Biocorp) and YPG (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 15 g/L agar) media plates, and described as an individual biotic effect. All the strains used in this study are deposited in the Department of Mycology and Genetics, Institute of Genetics and Microbiology, University of Wrocław, Poland. Two rye isolates of *E. nigrum*, UP_EPC_31 and UP_EPC_49 (accession numbers KM434173.1 and KM434171.1, respectively), and four species of dermatophytes, including *Trichophyton tonsurans* Malmsten, isolated from abdominal skin, *T. terrestris* Durie & D. Frey, isolated from soil, *T. mentagrophytes* (C. P. Robin) Sabour, isolated from ambulatory patient, and *Paraphyton cookei* Ajello KU687323.1, isolated from cave soil, were tested for the interspecies interactions. The fungal inoculates of ca. 4 mm diameter
were taken from the ten days old cultures on PDA, and then the mycelium was placed downwards and 2 cm apart in the center of the PDA and YPG plates. Each combination was prepared in four replicates.

Additionally, plates with mycelium of a single fungal species were used as a reference. After inoculation, the plates were incubated in the dark at 24 ± 0.5 °C. Biotic effects of the fungi in the combined cultures were evaluated after 10 days of growth. While evaluating the biotic effects, the surrounding area of one colony that was captured by another fungal species was observed, and then the occurrence of inhibition zone between the two colonies, as well as the reduction in the colony size were considered. The appearance of each effect was scored, and points were summarized according to the scale described by Mańka (1974), and the results are presented in Table 1. The biotic effect induced by a particular fungal species was evaluated as an individual biotic effect (IBE). The positive effect indicates the suppression of pathogen growth, and the negative effect indicates the lack of growth suppression. The effect might be scored with the value of 0, which indicates neutral influence (Mańka, 1974; Ogórek & Pląskowska, 2011). The size of the zone formed by *E. nigrum*’s colored metabolites and the size of the inhibition zones created by different *E. nigrum* isolates were measured. Each measurement was performed in four replicates.

**Table 1** The scale of scoring the biotic effects of the *Epicoccum nigrum* colony on the dermatophyte colony.

| Petri dish appearance | Points |
|-----------------------|--------|
| 0 | Both fungal colonies straightly abut to each other | 0 |
| I | *Epicoccum nigrum* colony abut to the dermatophyte colony in a slightly curved manner, surrounding less than 1/3 of the tested colony | +1 |
| II | *E. nigrum* colony abut to the dermatophyte colony in a slightly curved manner, surrounding at least 1/3 but no more than 1/2 of the tested colony | +2 |
| III | *E. nigrum* colony abut to the dermatophyte colony in a slightly curved manner, surrounding less than 1/2 but no more than 2/3 of the tested colony | +3 |
| IV | *E. nigrum* colony abut to the dermatophyte colony in a slightly curved manner, surrounding at least 2/3 of the tested colony | +4 |
| V | Every mm of the inhibition zone between both colonies, caused by the *E. nigrum* colony | +1 |
| VI | Dermatophyte colony smaller by at least 1/3 but no more than 1/2 than the control colony grown on separate plate | +1 |
| VII | Dermatophyte colony smaller by at least 1/2 but no more than 2/3 than the control colony grown on separate plate | +2 |
| VIII | Dermatophyte colony smaller by at least 2/3 than the control colony grown on separate plate | +3 |
| IX | Undeveloped dermatophyte colony | +4 |

The points were given based on the appearance of both the colonies in a Petri dish, as described by Mańka (1974).

Additionally, the toxicity effect of *E. nigrum* filtrates was examined by using the *Galleria mellonella* larvae model. Sterile fungal filtrates derived from 14-day-old cultures were incubated at 25 ± 0.5 °C in Sabouraud dextrose broth (peptone 10 g/L and glucose 40 g/L). Thereafter, the caterpillars were treated with 40 µL of the sterile fungal filtrates. Inoculations were performed directly into the hemocoel via the prolegs, by injections using the insulin syringes with 26G needles (Fuchs et al., 2010). Injections were preceded by the disinfection of the puncture sites with 70% ethanol. The inoculations with phosphate-buffered saline (PBS) and Sabouraud dextrose broth were used as the experimental controls. After the injection, the larvae were incubated at 37 ± 0.5 °C, and the viability of the caterpillars was
letters mark the effect of media on these biotic effects within a given mark differences in the interaction between a particular 

For each variant of the experiment, means followed by the same letter are not statistically different at \( \alpha \leq 0.01 \).

3. Results

Overall, both the \( E. nigrum \) isolates showed a positive biotic effect towards the tested dermatophytes, with an exception of \( E. nigrum \) UP_EPC_31 in the coculture with \( T. terrestre \) on YPG (Table 2). The strongest biotic effect was observed for \( E. nigrum \) UP_EPC_31 against \( T. tonsurans \) on PDA (\( p_{T. tonsurans, T. mentagrophytes} = 0.003661 \)). The same trend was observed in the case of YPG (\( p_{T. tonsurans, T. mentagrophytes} = 0.009396 \)). In the case of \( E. nigrum \) UP_EPC_49, all the interactions were positive, but were not significantly different on both the media plates.

### Table 2: The individual biotic effect (IBE) between the strains of \( E. nigrum \) and dermatophytes after 10 days of combined growth on PDA and YPG media plates. The same experiment was performed in four independent replicates.

| Dermatophyte species                  | \( E. nigrum \) UP_EPC_31 PDA\(^1\) | \( E. nigrum \) UP_EPC_31 YPG | \( E. nigrum \) UP_EPC_49 PDA | \( E. nigrum \) UP_EPC_49 YPG |
|---------------------------------------|-------------------------------------|-----------------------------|----------------------------|-----------------------------|
| Paraphyton cookei                     | 5.00 ab\(^2\)                       | 1.50 abB                    | 5.00 aA                    | 2.50 aA                     |
| Trichophyton mentagrophytes           | 4.25 aB                             | 0.75 bB                     | 4.20 aA                    | 1.25 aB                     |
| Trichophyton terrestre               | 4.00 ba                             | –1.00 cB                    | 2.75 aA                    | 1.25 aA                     |
| Trichophyton tonsurans                | 7.00 aA                             | 2.50 aB                     | 3.75 aA                    | 3.00 aA                     |

\(^1\) PDA (potato dextrose agar), YPG (yeast extract peptone dextrose).

\(^2\) For each variant of the experiment, means followed by the same letter are not statistically different at \( \alpha \leq 0.01 \) according to Tukey's HSD test. Small letters mark differences in the interaction between a particular \( E. nigrum \) isolate and the individual dermatophytes species; they refer to column means. Capital letters mark the effect of media on these biotic effects within a given \( E. nigrum \) isolate and a given species of dermatophytes; they refer to row means.

The biotic effects were significantly stronger on the PDA plates in comparison to the YPG plates (Table 2). The effect of a culture medium was specifically observed for \( E. nigrum \) UP_EPC_31, for which all the interactions varied in a highly significant manner (\( p_{PDA, YPG} = 0.000327 \) for \( T. tonsurans \), \( p_{PDA, YPG} = 0.000349 \) for \( T. terrestre \), \( p_{PDA, YPG} = 0.000850 \) for \( T. mentagrophytes \), and \( p_{PDA, YPG} = 0.000643 \) for \( P. cookei \)). In the case of \( E. nigrum \) UP_EPC_49, statistically significant differences between media were recorded only for \( T. mentagrophytes \) (\( p_{PDA, YPG} = 0.004612 \)) (Table 2).

The results of this study showed that the coculturing of one species with another species, as well as the culture medium, have an effect on the amount of pigments produced by \( E. nigrum \) and consequently, the appearance of inhibition zones (Table 3). All the tested dermatophytes stimulated \( E. nigrum \) UP_EPC_31 to secrete the colored substances on the PDA plates, with the strongest effect observed for its coculture with \( T. tonsurans \) (\( p_{T. mentagrophytes, T. terrestre} = 0.002184 \)). In contrast, on the YPG plates, this isolate produced colored substances only when it was cocultured with \( P. cookei \). Surprisingly, \( E. nigrum \) UP_EPC_49 always synthesized the pigments during the coculture with different dermatophytes, regardless of the medium. Moreover, there was no significant effect of the dermatophyte species on the synthesis of these colored substances by \( E. nigrum \) UP_EPC_49 on the YPG media, whereas on the PDA media, this isolate was highly stimulated by \( T. terrestre \) (\( p_{T. terrestre, T. tonsurans} = 0.00670 \)) to produce the pigments. There was also a significant impact of the media on the amount of secreted pigments by \( E. nigrum \) UP_EPC_31 (\( p_{PDA, YPG} = 0.000248 \) for \( P. cookei \), \( p_{PDA, YPG} = 0.000291 \) for \( T. mentagrophytes \), \( p_{PDA, YPG} = 0.000488 \) for \( T. terrestre \), and \( p_{PDA, YPG} = 0.000385 \) for \( T. tonsurans \)), as well as by \( E. nigrum \) UP_EPC_49 (\( p_{PDA, YPG} = 0.000291 \) for \( P. cookei \), \( p_{PDA, YPG} = 0.000292 \) for \( T. mentagrophytes \), and \( p_{PDA, YPG} = 0.006348 \) for \( T. tonsurans \)), with an exception in the coculture of \( E. nigrum \) UP_EPC_49 with \( T. terrestre \). However, in the case of \( E. nigrum \) UP_EPC_31, the inhibition zones were only formed on PDA plates in the cocultures with \( P. cookei \) (Figure 1),
Table 3  The ability of *Epicoccum nigrum* isolates to synthesize colored metabolites and create inhibition zones after 10 days of combined growth with the dermatophytes. A (+) indicates the formation of an inhibition zone, and a (–) indicates the lack of such zones. The indicated values are the average of the values from four independent experiments.

| Dermatophyte species       | E. nigrum UP_EPC_31 | E. nigrum UP_EPC_49 |
|----------------------------|--------------------|--------------------|
|                            | Colored metabolite zone | Inhibition zone     | Colored metabolite zone | Inhibition zone |
|                            | (mm) | PDA | YPG | (mm) | PDA | YPG | (mm) | PDA | YPG |
| *Paraphyton cookei*        | 7.00  | abA | 1.00 | aB | + | – | 4.10  | bB | 7.50 | aA | + | + |
| *Trichophyton mentagrophytes* | 8.03  | aA | 0.00 | aB | – | – | 3.25  | bB | 7.00 | aA | + | – |
| *Trichophyton terrestre*   | 4.00  | bA | 0.00 | aB | – | – | 9.00  | aA | 7.45 | aA | + | – |
| *Trichophyton tonsurans*   | 8.18  | aA | 0.00 | aB | – | – | 5.11  | bB | 7.18 | aA | – | – |

1  PDA (potato dextrose agar), YPG (yeast extract peptone dextrose).
2  For each variant of the experiment, means followed by the same letter are not statistically different at $\alpha \leq 0.01$ according to Tukey’s HSD test. Small letters mark the effect of a given species of dermatophytes on the synthesis of color metabolites by a given *E. nigrum* within a particular medium; they refer to column means. Capital letters mark the effect of media on the synthesis of color metabolites by a given *E. nigrum* isolate in the combined growth with a given species of dermatophytes; they refer to row means.

Figure 1  An example of the inhibition zone created by the secondary metabolites secreted by *Epicoccum nigrum* UP_EPC_31 (left side of the plate) to the PDA medium after 10 days in the paired growth with *Paraphyton cookei* (right side of the plate); IBE = 5.00.

whereas *E. nigrum* UP_EPC_49 formed the inhibition zones in a coculture with all the dermatophytes on PDA plates (besides *T. tonsurans*), and only with *P. cookei* on the YPG plates (Table 3).

The experiments performed to test the safety of *G. mellonella* larvae against the *E. nigrum* filtrates showed that the survival of larvae after 168 hr of incubation with the medium and the *E. nigrum* UP_EPC_31 filtrate decreased up to 96.7%, and in the case of *E. nigrum* UP_EPC_49, it decreased up to 95% (Figure 2). Since both, the medium and the secondary metabolites secreted by *E. nigrum*, reduced the survival rate of *G. mellonella* larvae to a similarly extent, this reduction in the viability is attributed to the medium, and not to the fungal filtrates.

4. Discussion

*Epicoccum nigrum* Link is a cosmopolitan fungus frequently isolated from plants, soil, or water, and is a well-known producer of various secondary metabolites (Sun et al., 2011). The results obtained in our studies confirm the potential application of this species as a biocontrol agent with antimicrobial properties. The antibacterial activity of the secondary metabolites produced by *E. nigrum* is well documented (Baute et al., 1978; Perveen et al., 2017). Moreover, antifungal properties of this...
species were also proved against numerous plant, animal, and human pathogens (Mallea et al., 1991). In the present study, we demonstrated the antifungal potential of *E. nigrum* against dermatophytes, since inhibitory biotic interactions were observed between the *E. nigrum* isolates and the dermatophytes, including *P. cookei*, *T. terrestre*, *T. tonsurans*, and *T. mentagrophytes*.

As shown in this study, the type of culture medium is an important factor in estimating fungal interspecies interactions (Ogórek et al., 2016). PDA is a medium preferable for *E. nigrum*, whereas dermatophytes exhibit better growth on YPG, and thus there were some differences between the strength of the interactions that were dependent on the medium. *Epicoccum nigrum* is also a well-known producer of colored metabolites and some of them, such as prodiginine, exhibits antimicrobial properties (Perveen et al., 2017). In addition, there is a correlation reported between the secretion of pigments and epicorazine A and B, by this species (Baute et al., 1978). Since the zones created by colored substances and biotic effects were stronger on the PDA medium, we can speculate that medium composition plays an important role in stimulating interactions between *E. nigrum* and the dermatophytes. PDA is a carbon-rich medium but is deficient in other nutrients (unlike YPG). Such stress conditions might also stimulate the pigment production by *E. nigrum* (Pradeep et al., 2013), and thus these colored substances might enhance the inhibitory biotic effects towards the dermatophytes (Fatima et al., 2016). As previously reported in the literature, fungi of the genus *Epicoccum* can also secrete bioactive metabolites with cytotoxic properties, e.g., some terpene metabolites and epicoccamide D (Palacio-Barrera et al., 2019). However, in this research study, we could show that *E. nigrum* species probably does not produce any cytotoxic substances, since the culture filtrates from *E. nigrum* strains did not reduce the viability of *G. mellonella* larvae at a significant level.

In conclusion, this study is the first report describing about the antagonistic interactions between *E. nigrum* and dermatophytes (*P. cookei*, *T. terrestre*, *T. mentagrophytes*, and *T. tonsurans*), as well as the effects of its secondary metabolites on *G. mellonella*, which is an eukaryotic model organism. The results indicate towards the possible application of *E. nigrum* secondary metabolites for the treatment of skin dermatophytoses. Therefore, in the near future, further studies will help to isolate and identify different secondary metabolites and determine their fungicidal properties.

**References**

Achterman, R. R., Smith, A. R., Oliver, B. G., & White, T. C. (2011). Sequenced dermatophyte strains: Growth rate, conidiation, drug susceptibilities, and virulence in an invertebrate model. *Fungal Genetics and Biology, 48*(3), 335–341. [https://doi.org/10.1016/j.fgb.2010.11.010](https://doi.org/10.1016/j.fgb.2010.11.010)
Baute, M. A., Deffieux, G., Baute, R., & Neveu, A. (1978). New antibiotics from the fungus *Epicoccum nigrum*. I. Fermentation, isolation and antibacterial properties. *The Journal of Antibiotics (Tokyo)*, 31(11), 1099–1101. https://doi.org/10.7164/antibiotics.31.1099

Colavolpe, B., Ezquiaga, J., Maiale, S., & Ruiz, O. (2018). First report of *Epicoccum nigrum* causing disease in *Lotus corniculatus* in Argentina. *New Disease Reports*, 38, 6. https://doi.org/10.5197/j.2044-0588.2018.038.006

de Cal, A., Larena, I., Lián, M., Torres, R., Lamarca, N., Usall, J., Domenichini, P., Bellini, A., Eribe, X. O., & Melgarejo, P. (2009). Population dynamics of *Epicoccum nigrum*, a biocontrol agent against brown rot in stone fruit. *Journal of Applied Microbiology*, 106(2), 592–605. https://doi.org/10.1111/j.1365-2672.2008.04030.x

Fatima, N., Ismail, T., Muhammad, S. A., Jadoon, M., Ahmed, S., Azhar, S., & Mumtaz, A. (2016). *Epicoccum* sp., an emerging source of unique bioactive metabolites. *Acta Poloniae Pharmaceutica*, 73(1), 13–21.

Fávaro, L. C., Sebastianes, F. L., & Araújo, W. L. (2012). *Fávaro, L. C., Sebastianes, F. L., & Araújo, W. L. (2012).*

Fusima, N., Ismail, T., Muhammad, S. A., Jadoon, M., Ahmed, S., Azhar, S., & Mumtaz, A. (2016). *Epicoccum* sp., an emerging source of unique bioactive metabolites. *Acta Polonae Pharmaceutica*, 73(1), 13–21.

Fávaro, L. C., Sebastianes, F. L., & Araújo, W. L. (2012). *Epicoccum nigrum* P16, a sugarcane endophyte, produces antifungal compounds and induces root growth. *PLoS One*, 7, Article e36826. https://doi.org/10.1371/journal.pone.0036826

Fuchs, B. B., O’Brien, E., Khoury, J. B. E., & Mylonakis, E. (2010). Methods for using *Galleria mellonella* as a model host to study fungal pathogenesis. *Virulence*, 1(6), 475–482. https://doi.org/10.4161/viru.1.6.12985

Jorjão, A. L., Oliveira, L. D., Scorzon, L., Figueiredo-Godoi, L. M. A., Prata, M. C. A., Jorge, A. O. C., & Junqueira, J. C. (2018). From moths to caterpillars: Ideal conditions for *Galleria mellonella* rearing for in vivo microbiological studies. *Virulence*, 9(1), 383–389. https://doi.org/10.1080/21505594.2017.1397871

Kavanagh, K., & Sheehan, G. (2018). The use of *Galleria mellonella* larvae to identify novel antimicrobial agents against fungal species of medical interest. *Journal of Fungi*, 4(3), Article 113. https://doi.org/10.3390/jof4030113

Mallea, M., Pesando, D., Bernard, P., & Khoulalene, B. (1991). Comparison between antifungal and antibacterial activities of several strains of *Epicoccum purpurascens* from the Mediterranean area. *Mycopestologica*, 15(2), 83–88. https://doi.org/10.1007/BF00436796

Mańka, K. (1974). Zbiorowiska grzybów jako kryterium oceny wpływu środowiska glebowego na choroby roślin [Fungal communities as a criterion for estimating the effect of the environment on plant diseases]. *Zeszyty Problemowe Postępu Nauk Rolniczych*, 160, 9–23.

Mańka, K., & Mańka, M. (1992). A new method for evaluating interaction between soil inhabiting fungi and plant pathogens. *IOBC/WPRS Bulletin*, 15(1), 73–75.

Ogórek, R., & Płaskowska, E. (2011). *Epicoccum nigrum* for biocontrol agents in vitro of plant fungal pathogens. *Communications in Agricultural and Applied Biological Sciences*, 76(4), 691–697.

Ogórek, R., Višňovská, Z., & Tancinová, D. (2016). Mycobiotas of underground habitats: Case study of Harmancecká cave in Slovakia. *Microbial Ecology*, 71(1), 87–99. https://doi.org/10.1007/s00248-015-0686-4

Palacio-Barrera, A. M., Areiza, D., Zapata, P., Atehortua, L., Correa, C., & Penuela-Vasquez, M. (2019). Induction of pigment production through media composition, abiotic and biotic factors in two filamentous fungi. *Biotechnology Reports*, 21, Article e00308. https://doi.org/10.1016/j.btre.2019.e00308

Perveen, I., Raza, M. A., Iqbal, T., Naz, I., Sehar, S., & Ahmed, S. (2017). Isolation of anticancer and antimicrobial metabolites from *Epicoccum nigrum* endophyte of *Ferula sambul*. *Microbial Pathogenesis*, 110, 214–224. https://doi.org/10.1016/j.micpath.2017.06.033

Pradeep, S. F., Begam, S. M., Palaniswamy, M., & Pradeep, B. V. (2013). Influence of culture media on growth and pigment production by *Fusarium moniliforme* KUMBF1201 isolated from paddy field soil. *World Applied Sciences Journal*, 22(1), 70–77.

Qian, Y., Yu, H., He, D., Yang, H., Wang, W., Wan, X., & L, W. (2013). Biosynthesis of silver nanoparticles by the endophytic fungus *Epicoccum nigrum* and their activity against pathogenic fungi. *Bioprocess and Biosystems Engineering*, 36(11), 1613–1619. https://doi.org/10.1007/s00449-013-0937-z

Somjaipeng, S., Medina, A., & Magan, N. (2016). Environmental stress and elicitors enhance taxol production by endophytic strains of *Paecilomyces variabilis* and *Epicoccum nigrum*. *Enzyme and Microbial Technology*, 90, 69–75.

Sun, H. H., Mao, W. J., I, J. Y., Xu, J. C., Li, H. Y., Chen, Y., Qi, X. H., Chen, Y. L., Xu, J., Zhao, C. Q., Hou, Y. J., & Yang, Y. P. (2011). Structural characterization of extracellular polysaccharides produced by the marine fungus *Epicoccum nigrum* JJY-40 and their antioxidant activities. *Marine Biotechnology (NY)*, 13(5), 1048–1055.

Weitzman, I., & Summerbell, R. C. (1995). The dermatophytes. *Clinical Microbiology Reviews*, 8(2), 240–259. https://doi.org/10.1128/CMR.8.2.240