JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

THE ENDOPHYTIC FUNGUS *EPICOCCUM NIGRUM*: ISOLATION, MOLECULAR IDENTIFICATION AND STUDY ITS ANTIFUNGAL ACTIVITY AGAINST PHYTOPATHOGENIC FUNGUS *FUSARIUM SOLANI*

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https://doi.org/10.55251/jmbfs.10093

ARTICLE INFO

Received 20. 4. 2023
Revised 1. 2. 2024
Accepted 5. 2. 2024
Published 1. 4. 2024

Regular article

OPEN ACCESS

ABSTRACT

Fusarium solani is a soil-borne fungus that causes fusarium dry rot disease in Upper Egypt. In this study, endophytic fungi from the Helwan University campus were collected, separated, and classified according to their morphological traits. Based on morphological and molecular characteristics, *Epicocum nigrum* was isolated and identified from *E. milii* leaves; other endophytic fungi were recovered and recognised morphologically from *E. hirta* leaves. The endophytic isolates were examined for their antifungal activity against *F. solani* on PDA plates using the dual culture technique, which exhibited a variable growth inhibition percentage. *E. nigrum* showed a highly inhibited percentage (94%) after 5 days. As well, *E. nigrum* metabolites were extracted by different solvents (e.g., ethyl acetate, dichloromethane, and chloroform: methanol, 2:1), and the crude extracts were active against *F. solani* compared to the control. The antifusarial activity was mostly reported in the ethyl acetate extract. Both light microscopy and scanning electron microscopy showed that higher concentrations of ethyl acetate extract caused a change in hyphal aggregation and spore formation in *F. solani*. Identification of antifungal components in ethyl acetate extract using GC-MS analysis revealed the presence of 2, 2, 3, 3, 4, 4-Hexadeutero Octadecanal, 2, 2-Dideutero Octadecanal, and Isochiapin B as major components that could have antifungal and antimicrobial substances. These results suggest using *E. nigrum* extracts for controlling phytopathogenic *F. solani*.

Keywords: Antifungal activity, phytopathogenic, GC/MS analysis, molecular identification, and isolation

INTRODUCTION

Every year, very large amounts of food produced are lost qualitatively and quantitatively due to plant diseases mainly caused by fungi, bacteria, nematodes, and parasitic plants; 85% of them are known as fungi (**Oerke et al., 1994**). The employment of chemical approaches is much more expensive, not ecologically sound, induces resistance in plant pathogens, generates environmental hazards, and most strikingly results in the accumulation of applied chemicals in crop products (**Vipin et al., 2021**). Biological control is a useful complement to agronomic control measures and a prospective substitute for chemical control. The use of antagonistic microorganisms as biological control agents for the management of soil-borne pathogens provides an ecologically friendly and pathogen-specific component of integrated disease management (**Arnold, 2007**).
The production of crops may be adversely impacted by severe plant diseases caused by phyto-pathogen fungus (**Doehlemann et al., 2017**). Ascomycota is the division to which Fusarium spp. belong. They can exist in both asexual and complete sexual stages. The discovery of a species' perfect state can result in a species receiving a new genus and species name, which may confuse the nomenclature (**Nelson et al., 1981**).
The most significant soil-borne fungal pathogen is *Fusarium solani*, which can grow in both cultivated and uncultivated soils and manifests as root rot and damping down in a variety of crop and vegetable plants (**Abu Taleb et al., 2011**). In Egypt, the potato has a significant position among all types of vegetable crops; in a range of 20% of the whole area dedicated to vegetable cultivation, potatoes are grown (**Kabeil et al., 2008**). Dry rot is a serious potato disease that is caused by Fusarium species and causes postharvest rotting as well as seed piece rot after sowing (**Du et al., 2012**). In the range of 6% to 25%, and on rare occasions losses of more than 60%, storage damages of potatoes caused by Fusarium dry rot have been noted (**Stefaczyk et al., 2016**). This genus contains numerous pathogen species that have an impact on numerous economically significant crops (**Aktaruzzaman et al., 2014**; **Hadieva et al., 2016**). The types of Fusarium spp. that infect potato tubers vary according to the screening period and location. According to **El-Hassan et al. (2007)**, *Fusarium solani* is the most frequent pathogenic species in Egypt. In addition to lowering crop yield, dry rot in potatoes can contaminate the tubers with mycotoxins, which can be consumed directly from Fusarium-infected plants or indirectly through the milk or meat of animals that have consumed contaminated feed and result in cyto-, geno-, neuro-, and hepatotoxic effects in both animals and humans. Beauvericin, moniliformin fumonisins, trichothecenes, and zearalenone are among the main mycotoxins generated by Fusarium spp., with the latter two being regarded as the most significant due to their frequent occurrence (**Nicholson et al., 2004**).
The third biggest genus of flowering plants according to **Govarts et al. (2000)** is Euphorbia L. (Euphorbiaceae). Since ancient and possibly even prehistoric times (**Pauketat et al., 2002**), the rich morphological variability and nearly global distribution of Euphorbia have drawn attention from people all over the world. A large variety of biological activities have been described for the genus Euphorbia, such as antiproliferative, multi-drug resistance-reversing, antimicrobial, vasoactive, immunomodulatory, anti-inflammatory, neuroprotective, and proinflammatory effects (**Shi et al., 2008**; **Vasas and Hohmann, 2014**). Endophytic fungi are microbes that live inside plant tissues without causing any symptoms to their host, and they are the most diverse life forms on earth (**Nisa et al., 2015**). According to earlier studies (Stierle and Stierle, 2015), endophytic fungi can create bioactive secondary metabolites that are similar to those of their host plant.
Epicoccum is an ascomycete, endophytic fungus that is often isolated at modest frequencies from various sources. *E. nigrum* is well known for its successful use in the bio-control of numerous phytopathogens (**Larena et al., 2005**; **Braga et al., 2018**) as well as for its capacity to produce a variety of classes of chemically, structurally, and biologically diverse secondary metabolites (**Fatima et al., 2016**; **Perveen et al., 2017**; **Larena et al., 2005**). In particular, *Botrytis cinerea* wax flower and *Claviceps africana* in sorghum (**Mohamed, 2015**; **Abdel Hafez et al., 2017**), *Epicoccum* spp., were demonstrated to be efficient biocontrol agents against a variety of phytopathogenic fungus. The action of *E. nigrum* against the plant pathogenic fungus *Fusarium solani*, however, has not been documented. The objectives of the current study were to: (1) identify the *E. nigrum* isolated from *E. milii* leaves based on morphological criteria and ITS sequences of rDNA data; and (2) evaluate its antifungal activity in addition to providing information relevant to the in vitro application of *E. nigrum* bioactive compounds as antifungal agents and biological approaches against the phytopathogen *Fusarium solani* and to achieve the goal of food security.

MATERIALS AND METHODS

The experiment was performed at the Mycology Lab, Department of Botany and Microbiology, Faculty of Science, Helwan University, Cairo, Egypt.

Collection of plants

I wanted to use the plants, both cultivated and uncultivated, on my university's campus to study the biological control effect of its endophytic fungus. So, a leaf of *Euphorbia milii* and samples of *Euphorbia hirta* were obtained in the autumn of 2021 (October) from the Helwan University Campus in Cairo, Egypt (Figs. 1a-1d). The laboratory processed the samples in 24 hours after receiving them in separate, clean plastic bags.

Figure 1 Collected plant samples from the Helwan University campus. *E. hirta* (a), *E. milii* (b), *E. milii* leaf samples (c) and *E. hirta* plant sample (d).

Isolation, purification of endophytic fungi from *E. milii* and *E. hirta*

Following the **Chi et al. (2019)** methodology with a minor modification, endophytic fungi were isolated from *E. milii* and *E. hirta*. To eliminate surface debris, leaves were washed under running water. Following a five-minute soak in 75% ethanol, they underwent a ten-minute soak in 10% sodium hypochlorite to be sterilized. After four rounds of sterile distilled water rinsing, each sample of leaves was then cut into small (0.5 cm) pieces with a sterile scalpel. The small samples of each part were put in the same Petri dishes (9 cm in diameter, and each piece was spaced by 1.5 cm) containing potato dextrose agar (PDA) (Fig. 3a, and 3b), composed of potato extract 200 g l^{-1}, glucose 10 g l^{-1}, and agar 16 g l^{-1} with antibiotics streptomycin (50 µgml^{-1}) and penicillin (100 µg ml^{-1}) to prevent any bacterial growth. Subsequently, the Petri plates were incubated at 25ºC in the dark and monitored every day for a month, to check the growth of endo-phytic fungal hyphae emerging from segments. After five days, the individual hyphal ends of the different fungi were taken off the agar plates and positioned on a fresh PDA medium for purification.
This medium was then incubated at 25°C for seven days. The final rinse water was spread on PDA plates and incubated at 28°C in the dark for seven days as the control to make sure that the surface sterilization had completely removed any epiphytic germs sticking to the segments externally.

Phytopathogenic fungus

The plant pathogenic fungus (*Fusarium solani*) used in this investigation was obtained from the agriculture research centre's culture stock at Giza, Egypt's plant pathology laboratory. On potato dextrose agar (PDA; BD, Sparks, MD) medium, *F. solani* was cultivated before being inoculated into Petri plates, which were subsequently incubated at 25 °C for five days.

Screening antifungal activity using the dual culture technique

We followed the procedure outlined by **Ali et al. (2015)** to test the antifungal activity of endophytic fungi isolated from *E. milii* or *E. hirta*. A mycelial disc (0.5 cm in diameter) taken from the periphery of a 7-day-old *F. solani* culture on PDA was injected in the plate's core and allowed to develop for three days at 25°C. The middle of the *F. solani* mycelial disc was surrounded by three regularly spaced 0.5-cm-diameter mycelial discs from endophytic fungal culture plates that were 7 days old.
For an additional 5 days, plates were incubated at 25°C. As a control, *F. solani* was employed but without any endophytic fungal inoculations. The growth inhibition equation was used to calculate the percentage of the pathogen's hyphal growth that was inhibited:

$$\%\boldsymbol{Inhibition} = \frac{C - \boldsymbol{T}}{C} \times \mathbf{100} \qquad \textbf{eq (1)}$$

Where T is the total growth of *F. solani* co-cultured with endophytic fungi and C is control, which is the total growth of *F. solani* alone.

Endophytic fungi identification

Based on the cultural and conidial traits, the mycological keys described by **Barnett and Hunter (1998)**, and the endophytic fungi spread out on slides, they were identified. Additionally, to corroborate the accuracy of the morphological identification of *Epicocum nigrum*, morphotypes were submitted to molecular identification techniques based on the Internal Transcribed Region (ITS) sequence analysis with PCR amplification (Figs. 6a–6c). The fungus isolate was grown on potato sucrose agar (PSA) medium and incubated for five days at 28°C (Pitt and Hocking, 2009). Intron Biotechnology Company's pathogen-spin DNA/RNA extraction kit (from Korea) was used to extract fungal DNA at the Molecular Biology Research Unit of Assiut University. The SolGent Company in Daejeon, South Korea, performed polymerase chain reaction (PCR) and sequencing. The universal primers ITS1 (forward) and ITS4 (reverse), which were added to the reaction mixture, were used to amplify the ITS region of the rRNA gene. The ITS1 (5' - TCCGTAGGTGAA CCTGCGG - 3') and ITS4 (5' - TCCTCCGCTTATTGATATGC -3') primers are composed as follows. With the identical primers and the addition of ddNTPs to the reaction mixture, the purified PCR product was sequenced (**White et al., 1990**). The Basic Local Alignment Search Tool (BLAST) available on the National Centre for Biotechnology Information (NCBI) website was used to examine the acquired sequences. MegAlign (DNA Star) software version 5.05. was used to analyze sequences and create phylogenetic trees.

Extraction of antifungal metabolites

Antifungal metabolites were isolated from *E. nigrum* cultures using a slightly modified **Kjer et al. (2009)** procedure. Conidial suspension was made by saturating an *E. nigrum* culture growing for 10 days with a sterile saline solution. In 250 ml Erlenmeyer flasks, one ml of the fungal spore suspension was mixed with 50 ml Potato Dextrose Broth (PDB) medium. Flasks containing culture media but lacking *E. nigrum* served as the controls. The flasks were incubated for 7 days at 250 °C under static circumstances, followed by 3 days of shaking at 100 rpm. Whatman No. 1 filter paper was used to separate the cultured broth. An equal volume of ethyl acetate, chloroform: methanol (2:1), and dichloromethane were mixed individually with the filtrate and control. The mixtures of culture broth and solvents were shaken for 30 minutes and then sat for 15 minutes until two separated layers had formed, Organic and aqueous fractions were separated using a glass separating funnel. The organic solvent layer and aqueous layer were collected. This step was repeated three times. The collected and extracted layers were pooled and evaporated using a vacuum rotary evaporator at 40°C.

Antifungal activity of *E. nigrum* extracts against *F. solani* (Bauer et al., 1966)

The disc diffusion technique experiment was used to assess the antifungal activity of extracts. The resulting dried extracts were dissolved in 1 ml of 100% methanol. Three 0.5 cm diameter paper discs (Whatman No. 1) saturated with 50 µl of the extracted compound or control cultural extracts were placed on 3 points of Petri plates containing Czapek Dox's medium (3% sucrose, 0.1% KH_2PO_4, 0.05% $MgSO_4$, 0.05% KcL, 0.3% $NaNO_3$, 0.001% FeSO4, and 1.6% Agar (**Thom and Raper, 1945**) inoculated with *F. solani* (3 days old) in the centre of medium plates. For 7 days, plates were incubated at 25°C. *F. solani*'s mycelial development was prevented in the direction of the extracts, indicating antifungal action.

The effect of antifungal EtOAc extract on the viability of *F. solani*

The following was the design of the experiment in a microtiter plate: F. solani spores (3×10^5/ml) were suspended in 1 millilitre of PDB. 50 µl of *F. solani* culture medium was added to the microtiter plate well, then inoculated with different concentrations (0.3–0.9%) of *E. nigrum* EtOAc extract, using *F. solani* culture medium only as a control (Fig. 2).

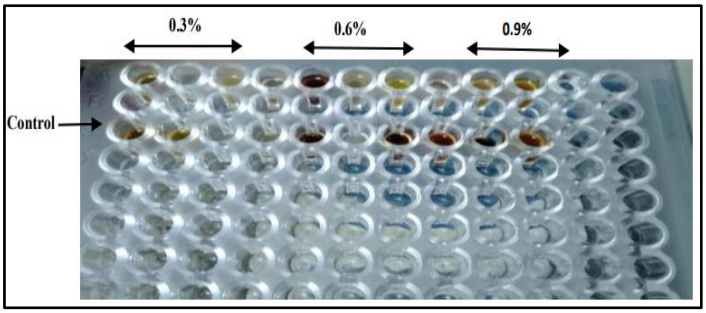

Figure 2 Microtiter plate after incubation for 48 h.

Triplicates of each concentration were used. The plate was incubated at 25°C for 48 h and then observed with a light microscope (Optika Microscope, Italy) or a scanning electron microscope. For scanning electron microscopy, the spores were fixed according to the protocol of **Kim et al. (2019)** and observed with a scanning electron microscope (Quanta FEG 250, FEI, USA) at the Desert Research Centre, Cairo, Egypt.

Chemical identification using gas chromatography-mass spectrometry (GC–MS) analysis

Samples (EtOAc extract) were performed using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness) at the Desert Research Centre, Cairo, Egypt. The components were identified by comparing their mass spectra and retention times to the mass spectral databases of NIST 11 and Wiley 09, respectively.

RESULTS

Isolation, purification of endophytic fungi from *E. milii* and *E. hirta*

After 5 days, *E. milii* and *E. hirta* provided 5 endophytic fungus isolates, which were identified as *Rhizopus oryzae*, *Alternaria alternata*, *Alternaria arborescens*, *Cladosporium cladosporioides*, and *Epicocum nigrum*. The first two endophytic fungi were found in the leaves of *E. milii*, and three more were found in the leaves of *E. hirta*. Table 1 shows the protocol used for surface sterilisation concludes the origin of fungal isolates (endophytic or phyllosphere) and lists the isolated fungi that were found in *E. milii* and *E. hirta* leaves that were gathered from the Helwan University campus. Endophytic fungal hyphae are visible arising from individual leaf segments (Figs. 3c–3)h.

Table 1 The protocol used for surface sterilization concludes the origin of fungal isolates (endophytic or phyllosphere).

Tissue	Surface disinfection protocol	Plant species	Origin of fungal isolates	
			Endophytic	Phyllosphere
Leaves	leaves were washed under running water. Following a five-minute soak in 75% ethanol, they underwent a ten-minute soak in 10% sodium hypochlorite to be sterilized. After four rounds of sterile distilled water rinsing, each sample of leaves was then cut into small (0.5 cm) pieces with a sterile scalpel.	*E. milii*	*Epicocum nigrum*	-ve
		E. milii	*Alternaria alternata*	-ve
		E. hirta	*Cladosporium cladosporioides*	-ve
		E. hirta	*Alternaria arborescens*	-ve
		E. hirta	*Rhizopus oryzae*	-ve

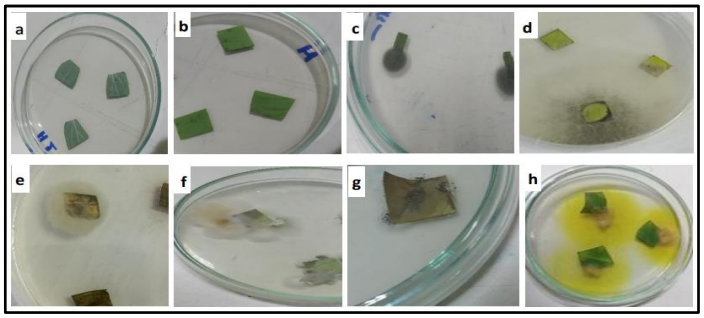

Figure 3 Isolation and purification of endophytic fungi. Leaf segments are placed on PDA plates (a, b). The endophytic fungal hyphae are emerging from leaf segments (c–h).

Screening antifungal activity using the dual culture technique

The results from Figs. 4a–4f and Fig. 5 indicate that all tested endophytic fungi inhibited the growth of *F. solani*. *Cladosporium cladosporioides*, *Rhizopus oryzae*, *Alternaria alternata*, *Alternaria arborescens* and *Epicocum nigrum* achieved inhibition percentages of 55, 60, 65, 75, and 94%, respectively, after 5 days. The maximum inhibition per cent (94%) of *F. solani* was recorded with *Epicocum nigrum*. So, *Epicocum nigrum* was selected for further investigations.

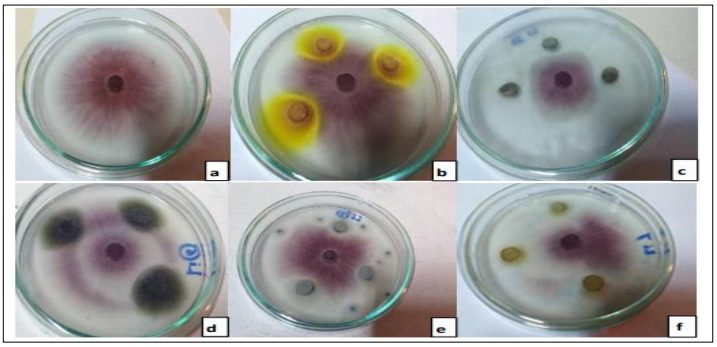

Figure 4 Screening antifungal activity using the dual culture technique. Phytopathogenic fungus *F. solani* (a). Endophytic fungi co-culture with *F. solani* (b-f).

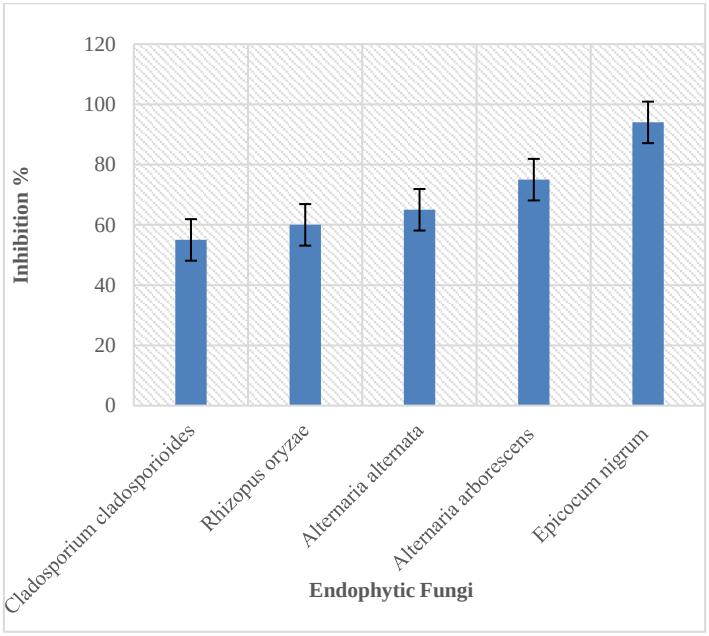

Figure 5 The tested endophytic fungi inhibited the growth of *F. solani*.

Endophytic fungi identification

Five endophytic fungi were identified at species level in the mycology laboratory at the Faculty of Science, Helwan University. The most antifungal activity, *Epicocum sp.*, was identified by molecular method to be *Epicoccum nigrum* AUMC15771(Fig. 6d).

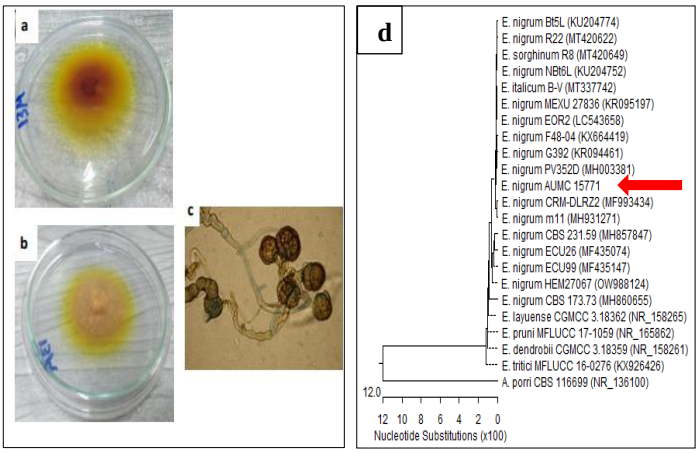

Figure 6 *E. nigrum* identification. Morphological growth of *E. nigrum* on PDA plates (a, and b). Microscopic examination of *E. nigrum* conidia (c). Phylogenetic tree based on ITS sequences of rDNA of the fungal sample isolated in the present study (*Epicoccum nigrum*, AUMC15771, arrowed) aligned with closely related strains accessed from GenBank (d) This strain showed 100% identity and 100% coverage with several strains of the same species. *Alternaria porri* was included in the tree as an outgroup strain. E = *Epicoccum*; A = *Alternaria. The accession* number of each fungal strain is written between parentheses.

Antifungal activity of *E. nigrum* extracts against *F. solani*

Antifungal activity was induced by the extracellular extract of *E. nigrum* or Czapeck Dox's medium extract. The extracts of these treatments were evaluated against *F. solani* mycelia using a filter paper disc diffusion assay. Results from these experiments showed that chloroform: methanol (2:1), dichloromethane, and the control cultural extract did not affect the inhibition of *F. solani* mycelia growth, whereas compared to ethyl acetate extract, a strong inhibition was observed for the growth of *F. solani* (4 cm inhibition zone) (Figs. 7a, and 7b). Thus, ethyl acetate extract was chosen for additional research.

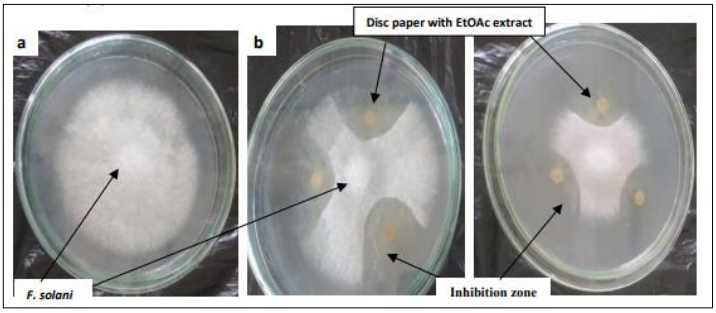

Figure 7 Antifungal activity of *E. nigrum* EtOAc extracts against *F. solani*. *F. solani* covered disc paper with the cultural extract(a). The extracellular *E. nigrum* extracts were compared to *F. solani* (b).

The effect of antifungal EtOAc extract on the viability of *F. solani*

To observe the effects of antifungal EtOAc extract on the viability of *F. solani* hyphae and spores, optical and electron microscopes were used. An obvious hyphal of *F. solani* was observed from the optical microscopy examination (Figs. 8a–8d), the normal morphological appearance of the control at zero concentration of the extracts revealing the highest growth of *F. solani*, and vice versa with the treatments, the hyphal growth, with condensed hyphal aggregations, and shorter hypha were decreased gradually with increasing the extract concentrations. Also, electron microscopy examination revealed a decrease in spore formation with an increase in the concentration of extract gradually from 0.3% to 0.9%, compared to the control spores that germinated normally (Figs. 8e–8h). From these examinations, we can expect that, morphologically, there will be a strong microscopic reduction in the mycelial growth and spore formation of *F. solani* in response to treatment with different concentrations of *E. nigrum* extracts.

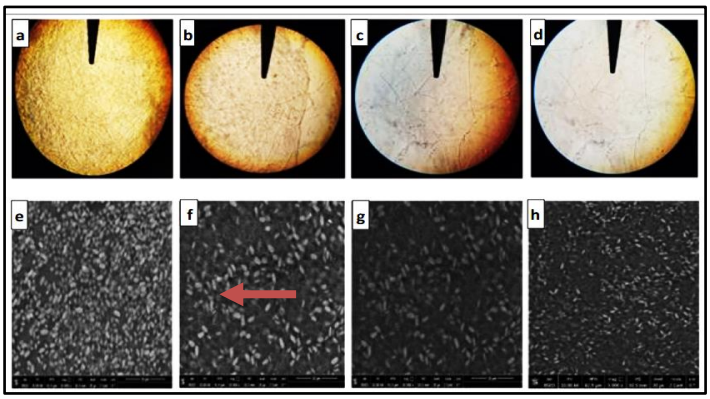

Figure 8 The effect of different concentrations of antifungal extract (0.3%, 0.6%, and 0.9%) on the viability of *F. solani*. The optical microscopical examination showed: control (8a), 0.3% (8b), 0.6% (8c), and 0.9% (8d). The electron microscopical examination: control (8e), 0.3% (8f), 0.6% (8g), and 0.9% (8h). The magnification is 1000x.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the antifungal *E. nigrum* EtOAc extract revealed that there are more than 12 compounds that were considered to be the major components. Among them, 2,2,3,3,3,4,4-Hexadeutero Octadecanal, 2,2-Dideutero Octadecanal, and Isochiapin B were identified chemically (Figs. 9a-9c), while the other compounds were fatty acids and their antifungal activity was not clear. So, these three compounds (2, 2, 3, 3, 4, 4-Hexadeutero Octadecanal, 2, 2-Dideutero Octadecanal, and Isochiapin B) could be active compounds that play antifungal activity against *F. solani*.

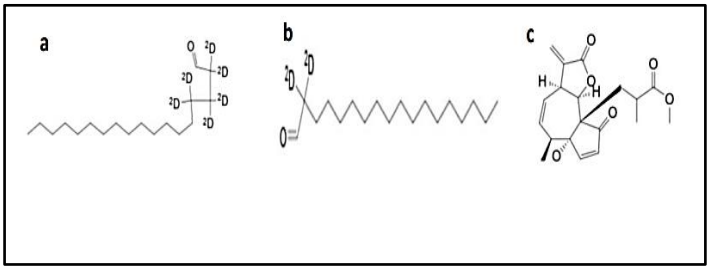

Figure 9 Chemical identification of antifungal *E. nigrum* ETOAc extract. 2, 2, 3, 3, 4, 4-Hexadeutero Octadecanal (a), 2, 2-Dideutero Octadecanal (b), and Isochiapin B (c).

DISCUSSION

Searching for eco-smart tools of biological origin for controlling plant microbial diseases is becoming a challenge for food safety and providing biocide-free food. Our study was based on the assessment of the antifungal activities of *Epicoccum nigrum* as an endophytic fungus isolated from *E. milii* leaves against the phytopathogenic fungus *Fusarium solani*. Endophytic fungus, in our research, belonged to fungi Ascomycota due to the absence of the known sexual state. It is also called dematiaceous fungus due to the dark colour of its cell walls, and it has also proven to be a potent biocontrol agent against many phytopathogenic fungi (**Peng et al., 2012**).

From the dual culturing technique, the isolated endophytic fungi revealed activity towards the *F. solani*. Furthermore, *E. nigrum* showed the highest inhibition per cent among five endophytic fungal isolates against *F. solani*. Thus, *E. nigrum* has been selected for further experimentation to explore the antifungal activity of its EtOAc extracts against *F. solani* using a disc diffusion assay. Our results were partially in line with the obtained results of **Adèle and Allison (2019)**, who isolated seven *Trichoderma* species from agricultural soil in the Annapolis Valley, NS, and identified them using ITS rDNA barcoding. Then, they noticed that in dual culture, all examined Trichoderma species displayed hostility towards FOC. This is also consistent with the findings of **Erfandoust et al. (2020)**, who investigated in vitro bioassays that revealed anti-Asperilli activity of the endophytic fungi in dual cultures and discovered that *Trichoderma koningii* CSE32 and *Trichoderma atroviride* JCE33 showed complete growth inhibition of both *Aspergillus niger* and *Aspergillus fumigatus*. Agar-diffusion assay results further revealed that the extracellular secondary metabolites (SMs) of four particular fungal endophytes had anti-Aspergillus activity at all treatment levels. In our research, a significant reduction in the spore formation and mycelial growth of *F. solani* was detected with the concentration of extracted compounds using electron and light microscopes. Thus, it was emphasised that *E. nigrum* EtOAc extracts had a significant effect on suppressing the growth of *F. solani*. The current findings resemble those of **Kim et al. (2019)**, who studied abnormal morphology caused by antifungal fractions and observed that electron micrographs revealed shrunken,

wrinkled spores and hyphae following treatment with the antifungal extracts, compared to the smooth surface of control spores. These findings imply a clear correlation between the structural damage to spores and mycelium and the antifungal fractions' ability to destroy them.

The EtOAc extract's GC-MS/MS analysis identified the following active chemicals as main constituents: Isochiapin B, 2, 3, 3, 4, 4-Hexadeutero Octadecanal, and 2, 2-Dideutero Octadecanal. This finding is consistent with literature by various researchers, such as **Kim et al. (2019)**, who found that the identification of antifungal agents in the active fraction using GC-MS analysis revealed the presence of cyclo-(Leu-Pro) and 9-octadecenamide as major components that have already been known as antifungal substances, and Twenty secondary metabolites, including thirteen known compounds and three new isocoumarin derivatives, pyrrolidinones, and one new pentaene diacid, was discovered by **Song et al. (2023)** while studying secondary metabolites from the endophytic fungus *Fusarium decemcellulare* F25 and their antifungal activities. Plant pathogen *C. musae* ACCC31244 was resistant to compounds 13, 14, and 17's antifungal effects. Also, **Prakash et al. (2021)** performed GC-MS analysis of the crude extracts of *Cystobasidium minutum* (BT-GA421), *Grammothele fuligo* (BT-BA212), and *Rigidoporus vinctus* (BT-GA2) and revealed the presence of phenol, 2, 4-bis (1,1-dimethyl ethyl)-, hexanedioic acid, bis (2-ethylhexyl) ester, and butylated Hydroxytoluene, respectively, and have antimicrobial activity.

This is the first report exploring the antifungal activity of the endophytic fungus *E. nigrum* isolated from *E. milii* against the fungus *F. solani*. Thus, *E. nigrum* in *E. milii* leaves could also be introduced as novel sources of bioactive compounds with antifungal activities against *F. solani* plant fungal pathogens.

CONCLUSIONS

Endophytic fungus *Epicocum nigrum* strain AUMC 15771 has antifungal activity for controlling *F. solani*, and here we show that culture extracts of this *E. nigrum* can be used for controlling the phytopathogenic fungus *F. solani*. We report for the first time the successful isolation and characterization of EtOAc extracts from *E. nigrum*. 2, 2, 3, 3, 4, 4-Hexadeutero Octadecanal, 2, 2-Dideutero Octadecanal, and Isochiapin B were extracted and identified chemically using GC/MS spectroscopy. Our results offer some indication that *E. nigrum* EtOAc extracts may provide a starting point for 2, 2, 3, 3, 4, 4-Hexadeutero Octadecanal, 2, 2-Dideutero Octadecanal, and Isochiapin B as antagonistic compounds against *F. solani*.

Acknowledgements: We appreciate Dr Mohamed Abo-Zaid, Professor of Plant Pathology, Agriculture Research Centre, Giza, Egypt, for the pathogen sample supply. Also, we appreciate Prof. Dr Abdel-Aziz Fayed, professor of taxonomy at Assuit University (Assiut Governorate, Egypt), for the identification of the collected plants.

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DECLARATIONS

Ethical approval: Not applicable

Author's contributions

Conceptualization, S.A.A., H.S.A., H.H.R., Y.B.S.; methodology, S.A.A.; formal analysis, S.A.A.; investigation, S.A.A.; data curation, S.A.A.; writing—original draft preparation, S.A.A., H.S.A., H.H.R., Y.B.S.; writing—review and editing and supervision, S.A.A. After reading the manuscript in its published form, each author has approved it.

Conflict of interest: The authors declare no conflict of interest.

Availability of data and materials: This published paper and its supplementary materials contain all the data created or analysed during this investigation.

Funding sources: This is a self-funded research work.

SUPPLEMENTARY MATERIALS

Molecular identification of *Epicocum nigrum*

Epicoccum nigrum AUMC15771 (517 letters)GCGGAAGGATCATTACCTAGAGTTTGTGGACTTCGGTCTGCTAC CTCTTACCCATGTCTTTTGAGTACCTTCGTTTCCTCGGCGGGTCCGCCC GCCGGTTGGACAACATTCAAACCCTTTGCAGTTGCAATCAGCGTCTGA AAAAACTTAATAGTTACAACTTTCAACAACGGATCTCTTGGTTCTGGC ATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGA ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTAT TCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCTTCAAGCTCTGC TTGGTGTTGGGTGTTTTGTCTCGCCTCCGCGCGCAGACTCGCCTTAAA ACAATTGGCAGCCGGCGTATTGATTTCGGAGCGCAGTACATCTCGCGC TTTGCACTCATAACGACGACGTCCAAAAGTACATTTTTACACTCTTGA CCTCGGATCAGGTAGGATACCCGCTGAACTTAAGCATATC

Table 2 GC-MS analysis of the antifungal *E. nigrum* EtOAc extract

RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
27.53	Hexadecanoic acid, methyl ester	6.73	10641385905.46	$C_{17}H_{34}O_2$	270
29.10 29.10	HEXADECANOIC ACID n-Hexadecanoic acid	1.52 1.52	2396725073.36 2396725073.36	$C_{16}H_{32}O_2$ $C_{16}H_{32}O_2$	256 256
30.70 30.70 30.70	9,12-Octadecadienoic acid (Z,Z)-, methyl ester 9-cis,11-trans-octadecadienoate ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE #	20.19 20.19 20.19	31911321579.91 31911321579.91 31911321579.91	C19H34O2 C19H34O2 C19H34O2	294 294 308
30.83 30.83 30.83 30.83 30.83	11-Octadecenoic acid, methyl ester 9-Octadecenoic acid, methyl ester, (E)- trans-13-Octadecenoic acid, methyl ester cis-13-Octadecenoic acid, methyl ester 9-Octadecenoic acid (Z)-, methyl Ester	29.17 29.17 29.17 29.17 29.17	46098319287.05 46098319287.05 46098319287.05 46098319287.05 46098319287.05	C19H36O2 C19H36O2 C19H36O2 C19H36O2 C19H36O2	296 296 296 296 296
30.92	9-Octadecenoic acid (Z)-, methyl Ester	1.06	1682605646.51	$C_{19}H_{36}O_2$	296
30.92 30.92	10-OCTADECENOIC ACID, METHYL ESTER 11-Octadecenoic acid, methyl ester	1.06 1.06	1682605646.51 1682605646.51	$C_{19}H_{36}O_2$ $C_{19}H_{36}O_2$	296 296
31.24 31.24 31.24 31.24 31.24	OCTADECANOIC ACID, METHYL ESTER Methyl stearate OCTADECANOIC ACID, METHYL ESTER OCTADECANOIC ACID, METHYL ESTER Methyl stearate	4.57 4.57 4.57 4.57 4.57	7229712099.37 7229712099.37 7229712099.37 7229712099.37 7229712099.37	C19H38O2 C19H38O2 C19H38O2 C19H38O2 C19H38O2	298 298 298 298 298
31.62 31.62 31.62 31.62 31.62	HI-OLEIC SAFFLOWER OIL 7-Methyl-Z-tetradecen-1-ol acetate 5,8-OCTADECADIENOIC ACID, METHYL ESTER 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester, (Z,Z,Z)- 9,12,15-OCTADECATRIENOIC ACID, 2-(ACETYLOXY)-1-[(ACETYLOXY)METHYL]ETHYL ESTER, (Z,Z,Z)-	0.52 0.52 0.52 0.52 0.52	822957291.60 822957291.60 822957291.60 822957291.60 822957291.60	C21H22O11 C17H32O2 C19H34O2 C25H40O6 C25H40O6	450 268 294 436 436
32.39 32.39 32.39	9,12-Octadecadienoic acid (Z,Z)- 9,12-OCTADECADIENOIC ACID (Z,Z)- 9,12-Octadecadienoyl chloride, (Z,Z)-	5.64 5.64 5.64	8918994228.59 8918994228.59 8918994228.59	C18H32O2 C18H32O2 C18H31ClO	280 280 298
32.39 32.39	(9E,12E)-9,12-OCTADECADIENOYL CHLORIDE # 17-Octadecynoic acid	5.64 5.64	8918994228.59 8918994228.59	C18H31ClO C18H32O2	298 280
33.49 33.49 33.49 33.49 33.49	9-OCTADECENOIC ACID (Z)- Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate 12-Methyl-E,E-2,13-octadecadien-1-ol 9-Hexadecenoic acid 9-HEXADECENOIC ACID	0.53 0.53 0.53 0.53 0.53	843365115.92 843365115.92 843365115.92 843365115.92 843365115.92	C18H34O2 C16H28O3 C19H36O C16H30O2 C16H30O2	282 268 280 254 254
34.17 34.17 34.17 34.17 34.17	9-OCTADECENOIC ACID (Z)- cis-13-Eicosenoic acid cis-11-Eicosenoic acid 12-Methyl-E,E-2,13-octadecadien-1-ol OXIRANEOCTANOIC ACID, 3-OCTYL-, CIS-	0.40 0.40 0.40 0.40 0.40	633722889.51 633722889.51 633722889.51 633722889.51 633722889.51	C18H34O2 C20H38O2 C20H38O2 C19H36O C18H34O3	282 310 310 280 298
34.63 34.63 34.63 34.63	2,2,3,3,4,4 HEXADEUTERO OCTADECANAL 2,2-DIDEUTERO OCTADECANAL ISOCHIAPIN B ISOCHIAPIN B %2<	0.59 0.59 0.59 0.59	929614098.56 929614098.56 929614098.56 929614098.56	C18H30D6O C18H34D2O C19H22O6 C19H26O6	274 270 346 350
34.63	DOTRIACONTANE	0.59	929614098.56	C32H66	450
36.36 36.36 36.36 36.36 36.36	9-OCTADECENOIC ACID (Z)- 12-Methyl-E,E-2,13-octadecadien-1-ol 9,12-Octadecadienoyl chloride, (Z,Z)- (9E,12E)-9,12-OCTADECADIENOYL CHLORIDE # Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	1.79 1.79 1.79 1.79 1.79	2826169339.53 2826169339.53 2826169339.53 2826169339.53 2826169339.53	C18H34O2 C19H36O C18H31ClO C18H31ClO C16H28O3	282 280 298 298 268
38.15 38.15 38.15 38.15 38.15	BIS(2-ETHYLHEXYL) PHTHALATE Diisooctyl phthalate Bis(2-ethylhexyl) phthalate Bis(2-ethylhexyl) phthalate 1,2-BENZENEDICARBOXYLIC ACID	16.40 16.40 16.40 16.40	25925082888.94 25925082888.94 25925082888.94 25925082888.94 25925082888.94	C24H38O4 C24H38O4 C24H38O4 C24H38O4 C24H38O4	390 390 390 390 390