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Original Research Article

Antagonistic Effect of Endophytic Fungi Isolated from *Nerium olender* against *Rhizoctonia solani*

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Abstract

Objective: To investigate the population of endophytic fungi accompanying to *Nerium olender* plant. ***Materials and Methods***: The study was conducted on three different sites in two governorates for three consecutive months with five samples from each site. The identification of the fungi was carried out using microscopic and molecular methods while the isolation, identification of fungal pathogen and preparation of aqueous extract were also successfully done. ***Results***: 14 species of fungi (*Penicillium notatum, Rhizopus nigricans, Rhizopus stolonifera, Fusarium Solani, Fusarium oxysporum, Alternaria sp., Cladosporium sp .and Curvularia sp.), w*hile the other sex species were identified by DNA investigation method and where (*Aspergillus* spp. (40%), *Penicillium* spp. (20%), *Rhizopus* spp. (12%), *Fusarium* spp. (10%), *Alternaria* sp. (4%), *Mucor circinelloides* (4%), *Neurospora Crassa* (3%), *Cladosporium* sp. (1%) and *Curvularia* sp. (1%). ***Conclusions***: The highest percentage of inhibition was for fungi *Aspergillus Flavus*, *Penicillium commune* and *Mucor circinelloides* which amounted to 100% and from the third day of growth until the completion of the control dish, then *Aspergillus niger* and *Rhizopus stolonifera*, with a 90% on the seventh day of growth, and *Penicillium commune*, *Rhizopus nigricans* with a rate ranging between 82-68%, while the rest of the fungi showed an antagonistic effect, but with rates less than 50%.

Keywords: *Nerium olender, Rhizoctonia solani*, endophytic fungi, Antagonistic.

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INTRODUCTION

Nerium oleander belongs to the Apocynaceae family, is species that currently classified in the genus *Nerium*, it is commonly known as oleander, from its superficial resemblance to the unrelated olive Olea. It is widely cultivated and originated in Mediterranean region and also distributed in different geographical and ecological places. Various plants are tested for their antifungal efficiency under complementary and alternative medicinal approaches; one of this plant is *Nerium oleander* which is considered as the most important medicinal plants that have many symbiotic relationships with fungi which called endophytic fungi [1].

The endophytic fungi are a group of fungi that founds in living and internal tissues of plants without causing any negative effects, generally, the fungi is able of symptomless occupation and appear healthy plant tissue. Important discoveries of natural products from endophytic fungi found in plants, representing a rich source of bioactive natural products which used in pharmaceutical and agricultural field, and a large number of secondary metabolites have been separate and distinguish from endophytic microbes and these are description with extensive references [2]. Some of these compounds are biologically active components and the compounds includes alkaloids, flavonoids, steroids, terpenoids, peptides, polyketones, quinols and phenols as well as some chlorinated compounds [3].

Fungal metabolites from endophytes greatly influence the biology of predators; many of experiments have been conducted by various research groups on the influence of endophyte infection of different grasses on fungal plant pathogens like *Rhizoctonia solani* which is considered as the most important species within the genus *Rhizoctonia*, is a soil borne plant pathogen with considerable diversity in cultural morphology, host range and aggressiveness, despite its history as a destructive pathogen of economically important crops worldwide [4].

The current work is to explore the population of endophytic fungi associated with the Nerium oleander plant. And test its activity *Rhizoctonia Solani*.

MATERIALS AND METHODS

Isolation of Endophytic Fungi

Three fresh parts of plant root, stem, leaf and rhizospheric soil, were used to isolate endophytic fungi:

- ✓ First step is surface sterilization by washing plant parts in tap water for 10min and transferred to sterilization [5].
- ✓ After sorting, selected samples were flooded in 75% ethanol for 1min, then submerged in 1.0% sodium hypochlorite (NaOCl) solution for 1min, then washed three times with distilled water and permitted to surface-dry on sterilized filter paper.
- ✓ Finally, sterilized samples were cut into 0.5 × 0.5cm pieces and placed them in Petri dishes containing water agar (WA) medium. Then the plant incubated at 25±2°C for 7 days for fungal growth. The successfully grown fungal isolates were purified by transferring into potato dextrose agar medium, many colonies appeared in one dish and with sub-cultivation was obtained pure genus. The process of transferring the fungal species to the culture medium was repeated several times for the purpose of obtaining pure cultures for each fungus as depicted by [6].

Identifications of Endophytic Fungi

The isolated fungi were identifying by two methods:

Identifications of Endophytic Fungi Microscopic Examination:

According to their Phenotypic and microscopic properties, and was depending on approved sources in the international classification of fungi like as explained by [7]. The percentage of isolates appearance was calculated according to the following equation.

$$Appeareance\ \% = \frac{The\ No.of\ times\ the\ fungus\ appeared\ in\ the\ samples}{Total\ No.of\ Samples}\ X\ 100$$

Identifications of Endophytic Fungi by molecular method:

DNA extraction, amplification and sequencing of fungal isolates

Genomic DNA (Deoxyribonucleic acid) of the isolated endophytic

DNA was extracted using Maxwell Blood DNA Kit (Model: AS1010, Promega, USA) according to manufacturer's protocol with some basic steps containing cell lysis, digestion of RNA (Ribonucleic acid) by RNase A, removal of precipitates and cell debris, DNA shearing, precipitation and purification.

Then extracted DNA samples were preserved in 50µl TE buffer at 4°C. PCR (Polymerase chain reaction) amplification process was carried out using HotStarTaq Master Mix Kit (QIAgen, USA). The universal primers ITS4

(5/-AGA GTT TGA TCM TGG CTC AG-3/) and ITS5

(5/-CGG TTA CCT TGT TAC GAC TT-3/) were used to amplify the ITS region of rDNA [8].

A 50µl reaction mixture containing 1µl template DNA (concentration 25–65ng/µl), 1µl of each primer (concentration 10–20 pMol), and 12.5µl of mastermix and the rest of water were used in PCR reaction. Reactions were performed for 30 thermal cycles following preheating at 95°C for 3min, denaturation for 30 s at 95°C, annealing at 48°C for 30 seconds, extension for 1min at 72°C and a final extension at 72°C for 5min. Purification of PCR product was then performed using 2% Agarose Gel Electrophoresis at 75V for 60min in 1× TAE buffer. 1% ethidium bromide was used for staining agarose gel and appropriate size 550bp stained fragment was collected from the agarose gel. PCR product purification was carried out using SV Gel and PCR Clean Up System (Promega, USA) following manufacturer's protocol. The amplified pure fungal DNA (PCR product) was sequenced using electrophoretic sequencing on an ABI370X1 DNA analyzer (Applied Biosystems, USA) using Big Dye Terminator v 3.1 cycle sequencing kit [9].

Isolation and Identification of fungal pathogen

Rhizoctonia solani that was isolated from infection potato root collected from many vegetable shops of Baghdad and Wasit and after transport root specimen to the laboratory in clean plastic bags, then immediately start sterilization protocol for isolating fungi as follows:

The roots were washed in running tap water and dipped in 70%ethanol for 10 seconds followed by immersion in sterile distilled water for 15 seconds and cut into segment (0.5-1cm), then transport in to PDA media that add antibiotic (to prevent growth bacteria) and incubate for 72hr. at 28±2°C, until fungi mycelium growth to make subculture, purification was on to new medium plates of (PDA) and incubated at 25°C for 2–3 days carried out by cutting a small piece of media with the tip of mycelium, and then transplanted days. Identify depended on key classification 3-6 Antagonism of Endophytic fungi against *Rhizoctonia solani* as explained by [10].

Preparation of the aqueous extract

Aqueous extracts were prepared according to [11] and the method was followed by mixing 20g, and the plant origin for each sample powder of distilled water with a capacity of 1000mL, the suspension was left in a shaking water bath at a temperature of 40°C for 24hours, and then filtered. It has been performed several times with a 0.22µm. Millipore filter the liquid is liquefied in sealed containers in the refrigerator at 4°C until use as prescribed by [12].

Preparation of the alcoholic extract

The preparation was based on previous study [13], Ethyl alcohol 95% was selected to prepare the alcoholic extract in the same way of prepare the aqueous extract.

Test the effect of plant extracts

The method of [13] was followed to test the effect of oleander parts on the growth of pathogenic fungi by mixing the dissolved aqueous extract after being sterilized and cooled at 50°C with liquid SDA medium, in concentrations (20, 15, 10mL) of extract in 100mL of nutritional medium, After solidification of the nutrient medium, a disc with a diameter of 6 mm of a growing fungal colony was placed on SDA medium for 7 days in the center of the dish containing one of the aforementioned concentrations.

Two types of comparison have been used:

Positive comparison: in which the antifungal, clotrimazole was added at a concentration of 2% to a plate containing SDA medium only.

Negative comparison: represents: an aqueous comparison that includes a dish containing only SDA medium without any other substance added. An alcoholic comparison that included a dish containing medium SDA and ethyl alcohol at the same concentrations previously mentioned. Positive and negative comparison dishes were grown with the same fungus in the same way and then all were incubated at a temperature of 25-28°C for a week to 10 days. To calculate the percentage of inhibition using the following equation [14]:

$$Inhibition\ \% = \frac{Average\ colony\ diameter\ in\ control\ plates - Average\ diameter\ in\ tretment\ \ plates}{Average\ colony\ diameter\ in\ comparison\ dishes}\ X\ 100$$

Antagonistic activity test

The inhibitory activity of the identified fungal species was measured by genetic and microscopic methods against the fungus that causes by root rot disease according to [15] modifying the dual-culture plate antagonism method, which states that the plate is divided into two parts. The fungal inoculum disc is placed on both sides of the plate, and then the plate is incubated at an appropriate temperature of 25ºC in the incubator. The measurement of colony growth is followed up and compared with the control dishes. Growth diameters were measured after 48hours, 72hours, five days and seven days, by which the fungal colony growth was completed in the control dishes, the treatment were replicated for three times for each fungal, and the experiment was repeated three times.

Statistical Analytics

The ready-made statistical analysis program SAS (System Analysis Statistical) was used (SAS, 2001) 0.05 level probability and on L.S.D. using averages and strengths, the treatments were compared with control dishes.

RESULTS

The results of isolations from plant parts showed a lot of variation and clear differences in isolated fungi numbers that varied between the parts and the highest numbers among the fungi were more present in the soil; however the fluctuation rates are shown in table 1.

Table 1: The yield of cultured fungi for the three months

Isolation Dtae	Rhizosphere	Root	Stem	Leaf	Total
October	214	92	94	75	475
November	164	177	134	109	589
December	168	161	140	170	639
Total	546	430	373	354	1703

The cultured fungi recorded different values for the three months and different locations. It was noted that the highest percentages were December, followed by November and, October due to climatic conditions. Also, the values were different between the plant parts (region).

Identification of Endophytic Fungi

The identification was carried out in two ways, the endophytic fungi can be identified using the sources approved in the International Classification of Fungi (the microscopic phenotypic), and the molecular method as shown in Table 2.

Table 2: The presence percent of fungal species and the approved classification method

Fungal Isolate	Direct examination	DNA Examination	Gene bank accession Number	Percentage %
Aspergillus Flavus		++	OM441939.1	25%
Aspergillus Terreus		++	MT530257.1	5%
Aspergillus Niger		++	OM017146.1	15%
Penicilliumommune		++	MT558930.1	10%
Penicillium notatum	++			10%
Neurospora Crassa		++	MT367687.1	3%
Rhizopus Stolonifera	++			8%
*Rhizopus*Nigricans	++			4%
Fusarium Solani	++			5%
Fusarium Oxysporum	++			5%
Mucor Circinelloides		++	MT603942.1	4%
Alternaria Sp	++			4%
Cladosporium sp	++			1%
*Curvularia*sp	++			1%

Isolation and identification of the pathogen

Three sites were used to take samples of potato tubers suspected of being infected with the fungus *Rhizoctonia solani*. All the sclerotia that found were taken, and as in the table (3) that shows the numbers of those sclerotia according to each site, and after the end of the incubation process, which lasted for a week under temperature (25ºC), the result was to obtain three isolates of *Rhizoctonia* sp. that were purified and classified according to the protocol:

Table 3: The numbers of sclerotia according to each site

Sample site	No. of sclerotia	No. of fungi colonies	*Rhizoctonia Occurrence*	*Rhizoctonia Frequency*
Baghdad 1	122	88	6	19%
Baghdad 2	153	109	22	35%
Wasit	85	71	17	21%

Test the effect of plant extracts

The method of Khanzada *et al*., 2006 [16] was followed to test the effect of oleander parts on the growth of pathogenic fungi by Two types of comparison have been used:

Positive comparison and negative comparison and calculate the percentage of inhibition using the following equation:

$$Inhibition\ \% = \frac{Average\ colony\ diameter\ in\ control\ plates - Average\ diameter\ in\ tretment\ \ plates}{Average\ colony\ diameter\ in\ comparison\ dishes}\ X\ 100$$

It is clear from the results that the efficiency of the alcoholic extract reached the highest activity against Rhizoctonia with diameter colonies of the fungi at Concentration%5 (v/v) for three parts plant extracts leaf, stems and root, respectively on (74.4,61.5,17.7) %, the inhibition loss reached, (14, 20, 57mm), while at a concentration of 10%, the average colonies diameters were (0,0 ,0.32 mm) and the percentage of Inhibition (100,100, ,84%) respectively, and at 15% and 20% concentrations, the percentage reached. For 100% inhibition, the average diameters of fungi were zero.

In the aqueous extract, the average diameters of the fungal colonies at the concentration 5%, were reached (50, 50,67) mm respectively and the inhibition percentage on (34.4,34.4,13.8) % and at a concentration of 10%, the rates of the diameters of the fungal colonies reached (10, 30, 49) mm, and the percentage of inhibition (78.9, 56.9, 33.94 %) respectively, and the percentage of inhibition was100% for the fungi at 20% concentration), Table 4.

Table 4: Effect of alcoholic and aqueous extracts of oleander plant parts on fungal growth (mm)

Extract Concentration	Rhizoctonia growth (mm)					
	Alcoholic extract			Aqueous extracts		
	Leaf	Stem	Root	Leaf	Stem	root
5	14	20	57	50	50	67
10	0	0	0.32	10	30	49
15	0	0	0	3	14	20
20	0	0	0	0	0	2
Control	90	85	85	90	90	89
L.S.D. 0.05	3.32					

Antagonism of Endophytic fungi against *Rhizoctonia solani:*

This activity was tested between fungi isolated from oleander against the pathogenic fungi isolated from infected potatoes, the culture dishes were inoculated with the two targeted fungi using the double culture technique on both sides of the dish, and the readings were taken after 48 hours, 72hours, five days, and seven days by which the control treatment was completed as in the Table 5 and Figure 1.

All fourteen isolates were tested for their antagonistic activity with *Rhizoctonia* to determine which had the highest inhibitory ability. The highest percentage of inhibition was for fungi *Aspergillus Flavus*, *Penicillium commune* and *Mucor circinelloides* which reached 100% and from the third day of growth and kept until the completion of the control dish, while the second were *Aspergillus niger* and *Rhizopus stolonifera*, with a rate of up to 90% on the seventh day of growth, and finally, in the third place was *Penicillium commune*, *Rhizopus nigricans* with a rate ranging between 68-82%,

Table 5: Antagonistic activity of endophytic fungi against *Rhizoctonia solani*

Fungal isolate	colony diameter and inhibition ratio of Rhizoctonia solani					
	5days	Inhibition %	7days	Inhibition %	Control	Inhibition%
Aspergillus flavus	0	100***	0	100***	9	0%
Aspergillus Terreus	3	66	3.6	60	9	0%
Aspergillus niger	1	92**	1.1	90**	9	0%
Penicillium commune	2	78*	2.4	82**	9	0%
Penicillium notatum	0	100***	0	100***	9	0%
Neurospor acrassa	4	55	5	48	9	0%
Rhizopus stolonifer	1	92**	1.5	90**	9	0%
Rhizopus nigricans	2.6	80*	3.1	68	9	0%
Fusarium solani	3.1	68	4.5	50	9	0%
Fusariumoxy sporum	4	54	5	48	9	0%
Mucor circinelloides	0	100***	0	100***	9	0%
Alternaria Sp.	4.5	50	5.6	40	9	0%
Cladosporium Sp.	6.3	30	7	23	9	0%
Curvularia Sp.	7.2	21	8	19	9	0%
L.S.D. 0.05	1.89					

Figure 1: Antagonistic effect of some endophytic fungi against rhizoctoniasolani

*A) Rhizopusstolonifer*against*Rhizoctoniasolani*

*B) Aspergillus Flavus*against*Rhizoctoniasolani*
*C) Fusarium oxysporum*against*Rhizoctoniasolani*
D) Penicillium commune against*Rhizoctoniasolani*
E) *Mucormcircinelloides*against*Rhizoctoniasolani*
*F) Fusarium Solani*against*Rrhizoctoniasolani*
*G) Mucormcircinelloides*controle
*H) Rhizopusstoloniferа*controle
*I) Rhizoctoniasolani*controle
*J) Fusarium Solani*controle

DISCUSSION

By reviewing these results, it was found that the surrounding soil with plant roots was the richest in terms of the number of isolated fungi. This may be explained by the fact that the soil is rich in plant residues and decomposing organic matter that helps the growth and spread of these fungi [17], the isolated fungi were 546 colonies with percentage about 32% in rhizosphere, followed by 430 colonies, at a rate of 25% in roots, while in stem were 373 colonies and in leaf were 354 colonies at percentages 22% and 21% respectively. These results were in agreement to was indicated by [17, 18], their findings shows that the different numbers and types of isolated fungi according to the different environmental conditions and the components of the isolated site [18].

Identification of Endophytic Fungi

The method of microscopic and morphological examination was used to identify a group of fungi isolated from the plant and they were eight fungi which were (*Penicillium notatum*, *Rhizopus nigricans*, *Rhizopus stolonifera*, *Fusarium Solani*, *Fusarium oxysporum*, *Alternaria sp.*, *Cladosporium sp .and Curvularia sp.)*, while the other sex species of endophytic fungi were identified by DNA investigation method and all PCR amplification products were sequenced and then searched in the Gene Bank database using BLAST tool (http//www.ncbi. nlm.nih.gov/BLAST/). The resultant sequences were aligned as Clustal W by using MEGA 7 software [19].

The table (3) shows the distinction of the second site of the city of Baghdad with the numbers of sclerotia and fungi isolated from it, which was Alwat Al-Rasheed, which is located southwest of Baghdad. The reason for this is that the potatoes were mostly imported and took a long time in the process of storage and transportation [20], and the difference in climatic conditions between the sites of cultivation and sale. According to the data mentioned in table 3, it was found that there are many fungi associated with stone bodies collected from infected plants, and there is no ability for all of those bodies to germinate and produce fungi again, due to many reasons such as, different conditions surrounding the presence of those bodies from one environment to another, as well as the pesticides used to combat this fungus from one farm to another, in addition to the presence of strong competitors from the accompanying fungi, which was stated by [21].

Test the effect of plant extracts

From the results of the statistical analysis, it was found that there were significant differences at the 0.05 probability level between the various extracts of oleander parts came in first place, the alcoholic extract followed by the aqueous extract in terms of inhibitory activity as shown significant differences, as the fungi were most affected by oleander leaf extracts.

As a result of the difference in the dielectric constant of the solvents used in the extraction, which affects the polarity of the solvent and its effectiveness against fungi due to the difference in the content of each extract of the active substances. This resulted in the superiority of the alcoholic extract over aqueous extract in preventing the growth of Fungi included, may be attributed to the oleander extracts containing compounds Toxic substances such as Oleandrin and Nerine, which are toxic even if there are traces of orchids which may dissolved in alcohol, thus affecting the fungi [15] as well as containing leaf oleander resins, flavonoids, tannins and glycosides the study with the findings of [23].

Antagonism of Endophytic fungi against *Rhizoctonia solani*

The result of the fungi showed an antagonistic effect, but with rates less than 50%, the high effectiveness of these fungi, is due to its production of many substances that have toxic effects on humans, animals, plants, as well as microorganisms, especially the fungal genera of Aspergillus and Penicillium, which secrete many mycotoxins such as aflatoxin, vioxantin, fumitoxin, ochratoxin and others [24].

The results in this study were identical to some extent with many studies and research that confirmed the active role of the fungi accompanying. The plant in an antagonistic is affected with the pathogenic fungi. Which could be due to many reasons, the most important of which is mainly the ability to compete with other fungi for food and carbon sources that they need because they are fast-growing and have the ability to cover a large surface area of food media very quickly. The other reason is due to the secretion by many of these fungi of active substances that have a direct inhibitory effect on many pathogenic fungi, as they act as anti-growth agents, including: phenolic compounds, alkaloids, peptides, steroids, as well as quinones and many enzymes Where studies indicate the role of these substances as

antioxidants and inhibitors of pathogens, as well as their role as antitumor [25].

CONCLUSIONS

- ✓ 14 different fungal genera were isolated successfully.
- ✓ The rhizosphere had the highest percentage of fungal colonies, followed by the root, stem, and leaves, respectively.
- ✓ *Aspergillus spp*. represents 40%, followed by the genus *Penicillium spp.* (20%), then *Rhizopus spp.* (12%), *Fusarium spp.* (10%), while *Alternaria sp.* (4%), *Mucor circinelloides* (4%), *Neurospora Crassa* (3%), *Cladosporium spand Curvularia sp.* were only (1%).
- ✓ The inhibitory activity exhibited significant differences between the alcoholic and aqueous extracts.
- ✓ The fungi were most affected by oleander leaf extracts.
- ✓ The highest percentage of inhibition was for Aspergillus *Flavus*, *Penicillium commune* and *Mucor circinelloides* which shows 100%.

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LIST OF ABBREVIATION

NaOCl	Sodium Hypochlorite
WA	Water Agar
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PDA	Potato Dextrose Agar
PCR	Polymerase chain reaction
SDA	Sabouraud Dextrose Agar
SAS	System Analysis Statistical

REFERENCES

1. Abo Nouh, F. A. (2019). Endophytic fungi for sustainable agriculture. *Microbial Biosystems*, *4*(1), 31-44. https://mb.journals.ekb.eg/article_38886.html
2. Akgun, S. G., Aydemir, S., Ozkan, N., Yuksel, M., & Sardas, S. (2017). Evaluation of the wound healing potential of Aloe vera-based extract of Nerium oleander. *North Clin Istanb*, *4*(3), 205-212.
3. Zuo, Y., Li, X., Yang, J., Liu, J., Zhao, L., & He, X. (2021). Fungal endophytic community and diversity associated with desert shrubs driven by plant identity and organ differentiation in extremely arid desert ecosystem. *Journal of Fungi*, *7*(7), 578.
4. Ajayi-Oyetunde, O. O., & Bradley, C. A. (2018). Rhizoctonia solani: taxonomy, population biology and management of rhizoctonia seedling disease of soybean. *Plant pathology*, *67*(1), 3-17. file:///C:/Users/User/Downloads/Ajayi-OyetundeandBradley2018.pdf
5. Soni, S. K., Singh, R., Ngpoore, N. K., Niranjan, A., Singh, P., Mishra, A., & Tiwari, S. (2021). Isolation and characterization of endophytic fungi having plant growth promotion traits that biosynthesizes bacosides and withanolides under in vitro conditions. *Brazilian journal of microbiology*, *52*, 1791-1805.
6. Durak, E. D. (2016, April). Biological control of Rhizoctonia solani on potato by using indigenous Trichoderma spp. In *AIP Conference Proceedings* (Vol. 1726, No. 1). AIP Publishing. https://pubs.aip.org/aip/acp/article-abstract/1726/1/020020/884232/Biological-control-of-Rhizoctonia-solani-on-potato?redirectedFrom=PDF
7. Padhi, L., Mohanta, Y. K., & Panda, S. K. (2013). Endophytic fungi with great promises: a review. *Journal of Advanced Pharmacy Education & Research*, *3*(3), 152-170. https://japer.in/storage/models/article/JOHVEyXHQVxLIqnbfflOFjPUEpXrvVfVbPWyiuo2RthbaPsc6wIx4YPEHISi/endophytic-fungi-with-great-promises-a-review.pdf
8. Hane, J. K., Anderson, J. P., Williams, A. H., Sperschneider, J., & Singh, K. B. (2014). Genome sequencing and comparative genomics of the broad host-range pathogen Rhizoctonia solani AG8. *PLoS genetics*, *10*(5), e1004281.
9. Poveda, J. (2021). Trichoderma as biocontrol agent against pests: New uses for a mycoparasite. *Biological Control*, *159*, 104634. https://www.sciencedirect.com/science/article/pii/S1049964421001043#:~:text=Indirect%20control%20of%20insects%20by,parasitism%20of%20insect%2Dsymbiotic%20microorganisms.
10. Rashmi, M., Kushveer, J. S., & Sarma, V. V. (2019). A worldwide list of endophytic fungi with notes on ecology and diversity. *Mycosphere*, *10*(1), 798-1079. https://www.mycosphere.org/pdf/MYCOSPHERE_10_1_19.pdf
11. Gupta, S., Chaturvedi, P., Kulkarni, M. G., & Van Staden, J. (2020). A critical review on exploiting the pharmaceutical potential of plant endophytic fungi. *Biotechnology advances*, *39*, 107462.
12. Hanif, S., Jabeen, K., Akhtar, N., & Iqbal, S. (2022). GC-MS analysis & antifungal activity of Datura metel L. against Rhizoctonia solani Kuhn. *Anais da Academia Brasileira de Ciências*, *94*(1), e20200851.
13. Wiyakrutta, S., Sriubolmas, N., Panphut, W., Thongon, N., Danwisetkanjana, K., Ruangrungsi, N., & Meevootisom, V. (2004). Endophytic fungi with anti-microbial, anti-cancer and anti-malarial

activities isolated from Thai medicinal plants. *World journal of microbiology and biotechnology*, *20*, 265-272. file:///C:/Users/User/Downloads/Endophytic_fungi_with_anti-microbial_anti-cancer_a.pdf

14. Zhang, N., Luo, J., & Bhattacharya, D. (2017). Advances in fungal phylogenomics and their impact on fungal systematics. *Advances in genetics*, *100*, 309-328.
15. Rustamova, N., Bozorov, K., Efferth, T., & Yili, A. (2020). Novel secondary metabolites from endophytic fungi: synthesis and biological properties. *Phytochemistry reviews*, *19*, 425-448. https://link.springer.com/article/10.1007/s11101-020-09672-x#citeas
16. Khanzada, S. A., Iqbal, S. M., & Akram, A. (2006). In vitro efficacy of plant leaf extracts against Sclerotium rolfsii Sacc. *Mycopathologia*, *4*(1), 51-53. http://pu.edu.pk/images/journal/impp/onlineconpdf/Vol.%204%20(1)%20%202006/MYCOPATH%2009.pdf
17. De Lamo, F. J., & Takken, F. L. (2020). Biocontrol by Fusarium oxysporum using endophyte-mediated resistance. *Frontiers in Plant Science*, *11*, 500488.
18. d'Errico, G., Aloj, V., Flematti, G. R., Sivasithamparam, K., Worth, C. M., Lombardi, N., ... & Vinale, F. (2021). Metabolites of a Drechslera sp. endophyte with potential as biocontrol and bioremediation agent. *Natural product research*, *35*(22), 4508-4516.
19. Escamilla, D., Rosso, M. L., & Zhang, B. (2019). Identification of fungi associated with soybeans and effective seed disinfection treatments. *Food science & nutrition*, *7*(10), 3194-3205.
20. Zhao, X., Song, P., Hou, D., Li, Z., & Hu, Z. (2021). Antifungal activity, identification and biosynthetic potential analysis of fungi against Rhizoctonia cerealis. *Annals of Microbiology*, *71*, 1-10. file:///C:/Users/User/Downloads/s13213-021-01654-4.pdf
21. Zhang, J., Liu, Y. X., Zhang, N., Hu, B., Jin, T., Xu, H., ... & Bai, Y. (2019). NRT1. 1B is associated with root microbiota composition and nitrogen use in field-grown rice. *Nature biotechnology*, *37*(6), 676-684.
22. Wang, G., Liu, Z., Lin, R., Li, E., Mao, Z., Ling, J., ... & Xie, B. (2016). Biosynthesis of antibiotic leucinostatins in bio-control fungus Purpureocillium lilacinum and their inhibition on Phytophthora revealed by genome mining. *PLoS pathogens*, *12*(7), e1005685.
23. Liu-Xu, L., Vicedo, B., García-Agustín, P., & Llorens, E. (2022). Advances in endophytic fungi research: a data analysis of 25 years of achievements and challenges. *Journal of Plant Interactions*, *17*(1), 244-266. https://www.tandfonline.com/doi/epdf/10.1080/17429145.2022.2032429?needAccess=true
24. Sarrocco, S., & Vannacci, G. (2018). Preharvest application of beneficial fungi as a strategy to prevent postharvest mycotoxin contamination: A review. *Crop protection*, *110*, 160-170. https://www.sciencedirect.com/science/article/abs/pii/S0261219417303411
25. Fontana, D. C., de Paula, S., Torres, A. G., de Souza, V. H. M., Pascholati, S. F., Schmidt, D., & Dourado Neto, D. (2021). Endophytic fungi: Biological control and induced resistance to phytopathogens and abiotic stresses. *Pathogens*, *10*(5), 570.