

Evaluation of the Antagonistic Potential of Endophytic Fungi from Sunflower, Olive, and Corn Leaves Against Plant-Pathogenic Fungi

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Abstract :

This study aimed to evaluate the antagonistic activity of endophytic fungi isolated from the roots, stems, and leaves of sunflower (*Helianthus annuus*) and olive (*Olea europaea*) against selected plant pathogenic fungi, including *Fusarium* sp., *Rhizoctonia solani*, and *Botrytis* sp.

The isolates were identified as *Phyllactinia* sp., *Aspergillus niger*, *Penicillium* sp., and *A. terreus*. Dual culture assays on potato dextrose agar (PDA) were conducted to assess their antagonistic potential. The highest inhibition rates against *Fusarium* sp. were recorded for *Phyllactinia* sp. (65.9%) and *A. niger* (64.5%), while *A. terreus* exhibited moderate inhibition (35.1%).

Moreover, the extract of *A. terreus* enhanced cucumber (*Cucumis sativus*) seed germination and reduced fungal infection caused by *Fusarium oxysporum*. These results highlight the potential of endophytic fungi—particularly *Phyllactinia* sp., *A. niger*, and *A. terreus* as promising biological control agents and sustainable alternatives to chemical fungicides in modern agriculture.

Keyword : Antagonistic, Endophytic, Sunflower, Olive, and Corn .

النشاط التضادي للفطريات المستنبطة المعزولة من أوراق عباد الشمس والزيتون والذرة ضد بعض الفطريات الممرضة للنبات أبراهيم بشار كاظم ، دينا يوسف محمد

مستخلص:

هدفت هذه الدراسة إلى تقييم النشاط التضادي للفطريات الداخلية المعزولة من جذور وسيقان وأوراق عباد الشمس (*Helianthus annuus*) والزيتون (*Olea europaea*) ضد بعض الفطريات الممرضة للنبات، بما في ذلك *Fusarium* sp. و *Rhizoctonia solani* و *Botrytis* sp.

تم تحديد العزلات على أنها *Phyllactinia* sp. و *Aspergillus niger* و *Penicillium* sp. و *A. terreus*. وقد أجريت اختبارات الزراعة المزدوجة على وسط آغار البطاطا الدكستروز (PDA) لتقييم قدرتها التضادية. وسُجّلت أعلى نسب تثبيط ضد *Fusarium* sp. لكل من *Phyllactinia* sp. بنسبة 65.9% و *A. niger* بنسبة 64.5%، في حين أظهر *A. terreus* تثبيطاً متوسطاً بلغت نسبته 35.1%.

علاوةً على ذلك، عزّز مستخلص *A. terreus* إنبات بذور الخيار (*Cucumis sativus*) وقلل من الإصابة الفطرية الناجمة عن *Fusarium oxysporum*. تُبرز هذه النتائج الإمكانيات الواعدة للفطريات الداخلية—وخاصة *Phyllactinia* sp. و *A. niger* و *A. terreus*—بوصفها عوامل مكافحة حيوية فعّالة وخيارات مستدامة بديلة عن المبيدات الكيميائية في الزراعة الحديثة.

INTRODUCTION

Endophytic fungi are microorganisms that live inside healthy plant tissues without causing harm. They promote plant growth, enhance nutrient uptake, and improve resistance to biotic and abiotic stresses by producing bioactive secondary metabolites, including alkaloids, phenolics, terpenoids, steroids, lactones, and quinones. These compounds exhibit diverse pharmacological activities, including antimicrobial, antioxidant, anticancer, antifungal, antiviral, and anti-inflammatory effects (Da Silva et al., 2022 and Nagaraju *et al.*, 2025).

Due to environmental and health concerns associated with chemical pesticides, there is increasing interest in biological control methods using endophytic fungi (Othman and Kakey, 2021). These fungi protect plants through mechanisms such as antibiosis, mycoparasitism, induced systemic resistance, and the production of growth-promoting hormones, including indole acetic acid and gibberellic acid (Tedsree *et al.*, 2022). Endophytic and rhizospheric microorganisms have

shown antagonistic effects against several phytopathogens, including *Fusarium* and *Rhizoctonia* species (Nagaraju *et al.*, 2025). For example, *Phyllactinia* sp. has been used as a biocontrol agent against *Rhizoctonia solani*, the pathogen responsible for the *Rhizoctonia–Meloidogyne* complex in chickpea (El-Nagar and Ghanem, 2024).

Biocontrol agents (BCAs), mainly bacterial and fungal isolates from the phyllosphere, endosphere, or rhizosphere, are effective alternatives for controlling plant diseases (Segaran and Sathiarely, 2019). In this context, exploring endophytic fungi from medicinal and oil-rich plants such as sunflower (*Helianthus annuus*) and olive (*Olea europaea*) is particularly valuable. These plants are rich in bioactive compounds and have been used for over 5,000 years in traditional medicine for their antimicrobial, anti-inflammatory, and antioxidant properties (Jadhar *et al.*, 2020).

The current study focuses on isolating and identifying endophytic fungi from sunflower and olive plants, evaluating their antagonistic activity against pathogenic fungi (*Fusarium sp.*, *Rhi-*

zoctonia solani, and *Botrytis sp.*), and assessing the effect of *Phyllactinia sp.* extract on cucumber seed growth and resistance to *Fusarium solani*. This research contributes to the development of eco-friendly, fungal-based biocontrol agents as sustainable alternatives to synthetic fungicides.

Materials and Methods

Isolate the endophytic fungi.

Endophenotypic fungi were isolated from the roots, stems, and leaves of sunflower and olive plants from Diwala Governor (33 ° 57 '59, N, 44 ° 54 '58 " E; decimal: 33.9664, 44,9161). According to Al-Nuaimy and Hawar (2024), the isolates were and they included the following species :*Phyllactinia sp*, *A.niger*, *A.terreus*, and *Penicillium*.

Collecting Plant-pathogenic fungi.

The Department of Plant Protection at the College of Agriculture, University of Baghdad, provided the recognized types of plant-pathogenic fungi (*Fusarium*, *Rhizoctonia solani*, and *Botrytis sp.*). The isolates were stored in PDA growth media in the refrigerator until needed.

Evaluation of the inhibitory ability of endogenous fungi extracted from the root, stem, and leaves of olive and sunflowers against plant pathogenic fungi using dual culture technology.

Using a sterile cork hole, a 5 mm diameter disc of fungal growth was extracted from 7-day-old fresh fungal cultures of each endophytic fungus and plant pathogen, isolated from the root, stem, and leaves of the olive plant. The two discs were placed 1 cm from the edge and approximately 6 cm apart on potato dextrose agar (PDA) medium in a single Petri dish. A comparison plate (control) containing only the pathogenic fungus was prepared.

The plates were incubated at a temperature of $28 \pm 2^\circ\text{C}$ for 7 days.

The diameter of the pathogenic fungus colony was measured (calculated as the average of two perpendicular diameters) across the different treatments.

The antagonistic activity of the isolated endophytic fungi was determined by assessing the rate of growth inhibition of the pathogenic fungus, using the following equation:

$$\text{Growth inhibition (\%)} = \frac{(D1 - D2/D1) \times 100}{D1}$$

Where:

D1 = The diameter of the pathogenic fungus colony alone (control).

D2 = The diameter of the pathogenic fungus colony in the dual culture (with the antagonistic fungus (Mishra et al., 2017)

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The antagonistic relationship between the endophytic and pathogenic fungi was examined to determine whether it manifested as growth inhibition (indicated by a clear inhibition zone between the colonies), overgrowth of one fungus on the other, or cessation of growth upon contact between the two. The outcomes of this assay were evaluated according to the growth inhibition scale ranging from 0 to 4, as described by (To et al., 2020) and illustrated below.

0 =No growth inhibition, 1 = 1-25% growth inhibition, 2 = 26-50% growth inhibition, 3 = 51-75% growth inhibition, 4 = 76-100% growth inhibition.

Evaluation of the Antifungal Activity of Endophytic Fungal Filtrates Against Selected Plant Pathogenic Fungi.

Endophytic fungal isolates exhibiting strong antagonistic activity were selected using the dual culture technique. These included *Phyllactinia sp.*, *Aspergillus niger*, *Penicillium chermesinum*, and *A. terreus*. Each 250 mL Erlenmeyer flask containing 150 mL of potato dextrose broth (PDB) was inoculated with two 5 mm agar plugs 28± 2°C for 7 days old fungal cultures and incubated with shaking at 125 rpm for 14 days. The fungal biomass was separated using Whatman filter paper, and the resulting filtrate was passed through a 0.22 µm Millipore membrane filter to remove spores, yielding a sterile, cell-free endophytic fungal filtrate (Kharwar et al., 2012).

● Assessment of Endophytic Fungal Filtrates Against Plant Pathogen Growth.

The endophytic fungal filtrates obtained from the previous step were incorporated into warm, sterile potato dextrose agar (PDA) at approximately 45–50°C to achieve a final concentra-

tion of 60%. The mixture was thoroughly mixed and then poured into sterile Petri dishes. Sterile PDA medium without filtrate served as the control. After solidification, each plate was inoculated at the center with a 5 mm diameter mycelial disc taken from the actively growing margin of the tested pathogenic fungi (*Fusarium sp.*, *Botrytis sp.*, and *Rhizoctonia solani*). The inoculated plates were incubated at 27°C for seven days.

The results presented in Table 1 indicate a variation in the antagonistic activity of the endophytic fungi isolated from sunflower and olive against the different plant pathogenic fungi. Among these isolates, *Phyllactinia spp.* and *A. niger* showed the strongest antagonistic effects against the pathogenic fungus *Fusarium*, with inhibition rates of 65.9% and 58.53%, respectively. The endophytic fungal species *Phyllactinia* exhibited the highest inhibition rate of 62.91%. In contrast, against the pathogenic fungus *R. solani*, the *A. niger* species demonstrated the greatest antagonistic activity, achieving an inhibition rate of 68.53%. In terms of antagonistic activity against the patho-

genic fungus *A. terreus*, most endophytic fungi displayed lower inhibition rates than those recorded against *Fusarium* and *R. solani* and *botrytis spp.* Among them, *A. terreus* and *A. niger* showed inhibition levels ranging from 35.10%.

The limited antifungal effect observed at low concentrations is consistent with findings by Adnani *et al.* (2024), who emphasized that many crude fungal extracts require purification or optimized extraction solvents to achieve strong antifungal activity. Meanwhile, the slight increase in radicle elongation at 25% concentration corresponds with evidence that low-dose fungal metabolites can trigger mild stress responses and IAA-associated growth pathways in early seedlings (Da Silva *et al.*, 2022).

Overall, the results support the classification of *Phyllactinia sp.* extract as a dual-function metabolite source, with strong antifungal activity at high concentrations and potential growth-promoting effects at lower doses. Further purification or fractionation is recommended to enhance its antifungal specificity (Anwar *et al.*, 2022).

Table 1. Endophytic fungi isolated from the roots, stems, and leaves of sunflower and olive, and their corresponding percentages of growth inhibition against plant-pathogenic fungi, as determined by the dual culture method on PDA medium incubated at $27 \pm 2^\circ\text{C}$ for 7 days.

Endophytic fungi isolates	<i>Fusarium</i>	<i>R.solni</i>	<i>Botyrtis spp</i>
<i>Phyllactina</i>	65.9±0.00	62.91±1.59	54.1±3.12
<i>Pencilium sp</i>	41.5±1.12	40.1±2.12	36.11±0.00
<i>A.niger</i>	64.53±0.00	58.53±0.00	38.12±1.00
<i>A.terrues</i>	35.10±2.12	31.22±1.22	35.10 ± 0.12

The antagonistic interactions between endophytic fungi isolated from sunflower and olive root, stem, and leaves and

plant-pathogenic fungi, as illustrated in Figures 1–3, demonstrate the occurrence of different forms of antagonism.

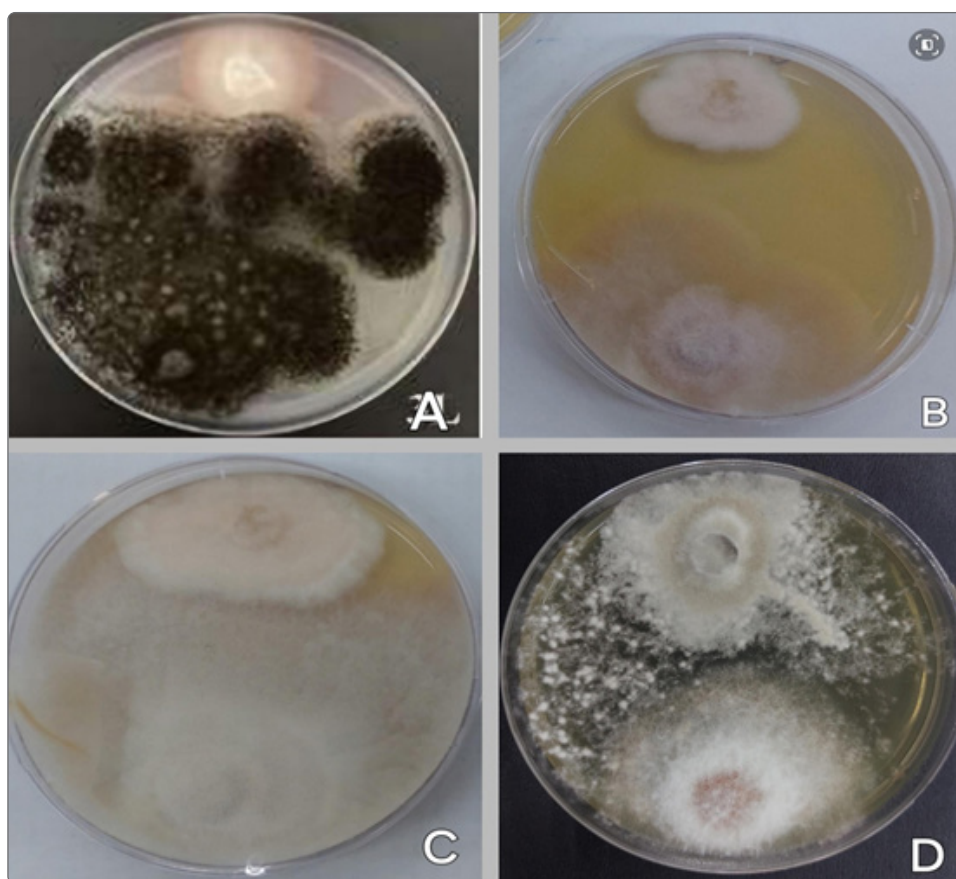


Figure 1. Antagonistic activity of endophytic fungi isolated from Aloe vera leaves against the pathogenic fungus *Fusarium* sp., evaluated by the dual culture method on PDA medium and incubated at $27 \pm 2^\circ\text{C}$ for 7 days. The pathogenic fungus appears in the upper part of the images. Description: (A) *A. niger* vs. *Fusarium* (B) *A. terreus* vs. *Fusarium* (c) *Phyllactina* sp vs. *Fusarium* (D) *Penicillium* vs. *Fusarium*.

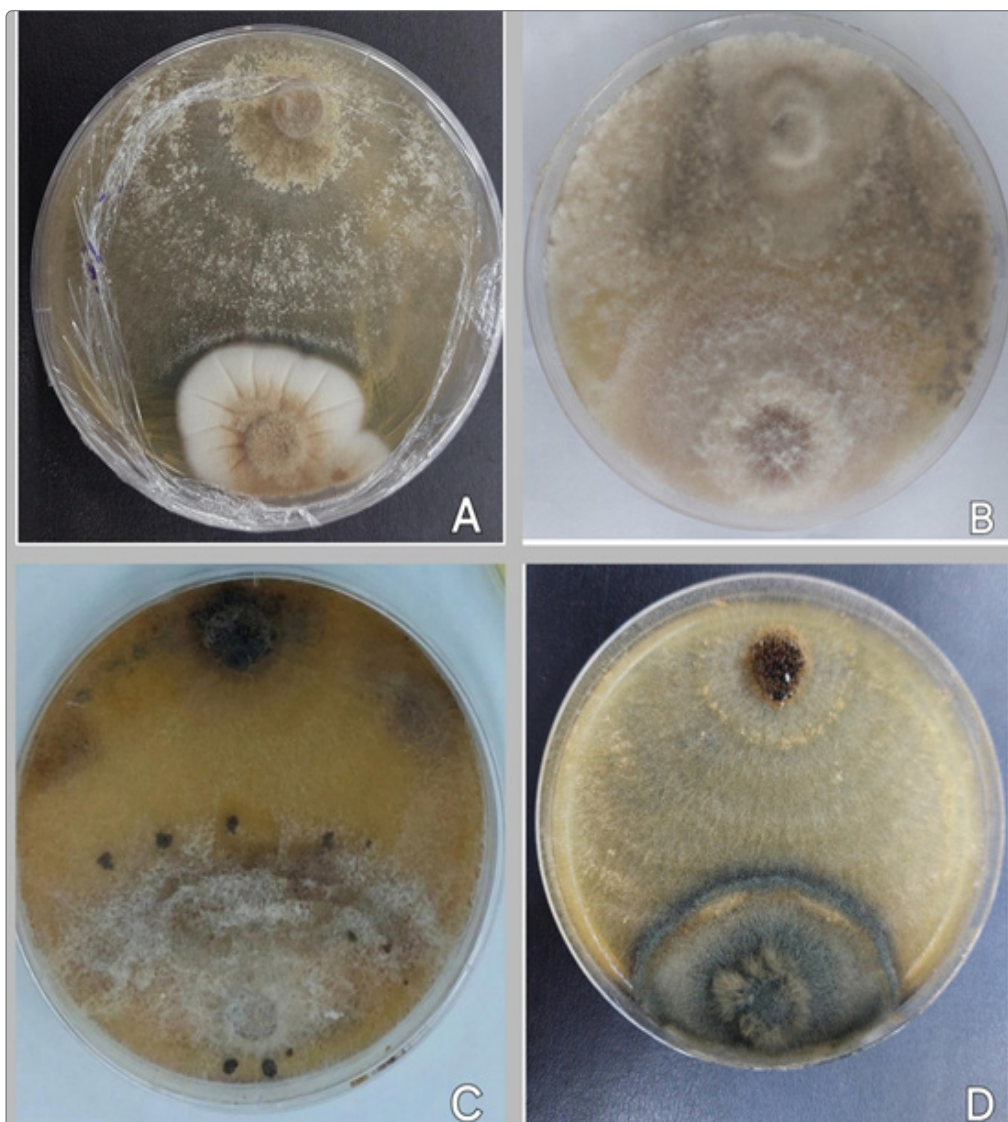


Figure 2: Antagonistic activity of endophytic fungi isolated from Aloe vera leaves against the pathogenic fungus *R.solani*, evaluated by the dual culture method on PDA medium and incubated at 27 ± 2 °C for 7 days. The pathogenic fungus appears in the upper part of the images: Description : (A) *A.terreus* vs *R.solani* (B) *Penicillium* sp vs *R.solani* (C) *Phyllactina* sp vs *R.solani* (D) *A.niger* vs *R.solani*.

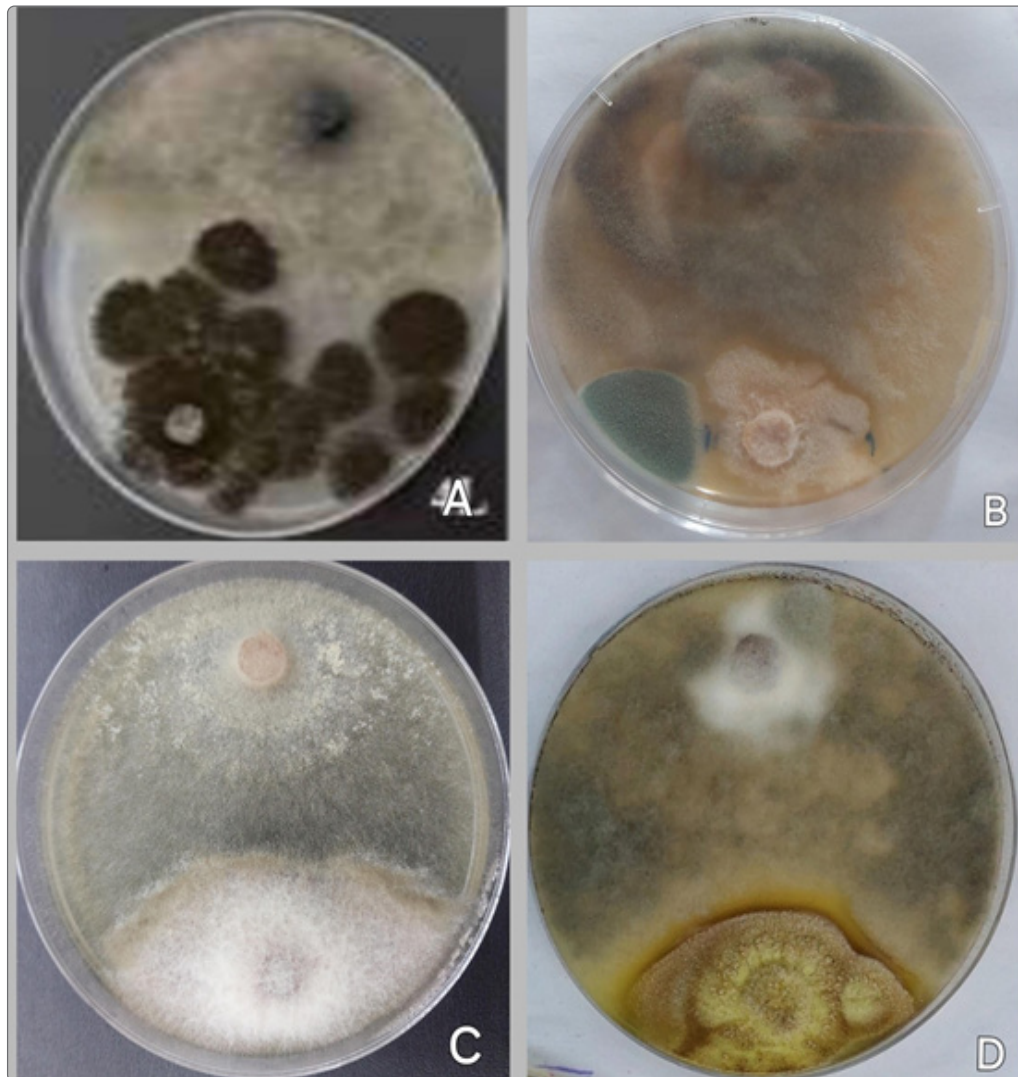


Figure 3: Antagonistic activity of endophytic fungi isolated from Aloe vera leaves against the pathogenic fungus *Botrytis* sp, evaluated by the dual culture method on PDA medium and incubated at $27 \pm 2^\circ\text{C}$ for 7 days. The pathogenic fungus appears in the upper part of the images: Description: (A) *A.niger* vs *Botrytis* sp (B) *Penicillium* sp vs *Botrytis* sp (C) *Phyllactinia* vs *Botrytis* sp (D) *A.terreus* vs *Botrytis* sp .

Testing the activity of *Phyllactinia* sp extract on cucumber seeds against *Fusarium oxysporum*.

The activity of *Phyllactinia* sp. extract against *Fusarium oxysporum*

showed a clear concentration-dependent pattern. The 100% extract fully inhibited fungal growth and reduced cucumber seed germination, while 50% and 25% concentrations caused

partial inhibition and induced moderate changes in radicle and secondary root growth.

This behavior aligns with recent studies showing that crude fungal metabolites often act as antifungal agents at high concentrations and plant growth promoters at lower doses. El-Nagar and Ghanem (2024) reported that crude extracts containing mixed secondary metabolites often exhibit a shift from stimulatory to inhibitory effects, depending on the concentration. Similarly, Nagaraju *et al.* (2025) demonstrated that compounds such as phenolics, lactones, and organic acids may enhance root development at sub-lethal levels but become phytotoxic at higher doses.

Testing the activity of *Phyllactinia* sp extract on cucumber seeds against *Fusarium oxysporum*.

The results of soaking cucumber seeds in *Phyllactinia* sp extract demonstrated variable growth responses depending on both the concentration and duration of exposure. As shown in Figure 4, seeds treated with 100% ethyl acetate crude extract exhibited pronounced growth inhibition when

soaked for periods exceeding 24 hours. Conversely, treatment with a 50% extract concentration promoted the formation of secondary roots, although this stimulatory effect diminished with longer soaking times of 48 and 72 hours (El-Nagar & Ghanem, 2024). At a lower concentration of 25%, the extract slightly enhanced radicle elongation and secondary root initiation in a small number of seeds after 24 hours, with only 3–4 seeds showing early signs of growth after 48 and 72 hours.

In vitro antagonistic assays further revealed that the *Phyllactinia* sp extract exhibited weak antifungal activity against *Fusarium* spp. when incorporated into agar media. This suggests that while certain active metabolites in *Phyllactinia* sp. may promote seed germination at low concentrations, their antifungal potential under the tested conditions appears to be limited (Adnani *et al.*, 2024). Overall, these results indicate that low concentrations of *Phyllactinia* sp extract may be more favorable for enhancing cucumber seed growth. In contrast, the observed weak antifungal effect could be attributed to the low concentration or purity of

bioactive compounds. Further optimization—such as employing alternative solvents or extraction techniques like

centrifugation may be required to obtain metabolites with stronger antifungal properties (Nagaraju *et al.*, 2025).

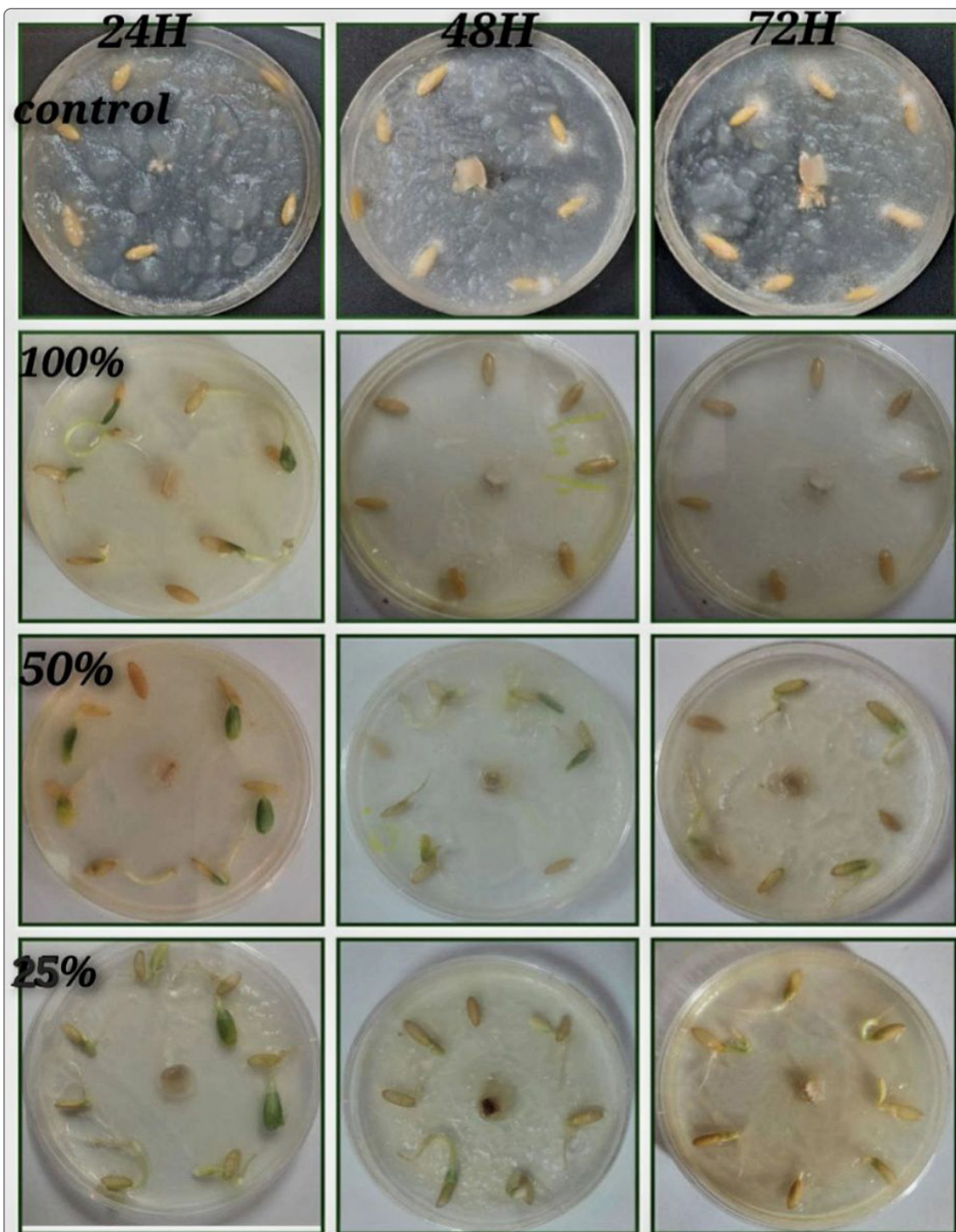


Figure 4: Testing of the active extract of *Phyllactina* sp in different concentrations against *Fusarium oxysporum*.

The results indicated that the extract of *Phyllactina* spp displayed potent antifungal activity in a concentration-dependent manner. Complete inhibition of *Fusarium* growth was achieved at 100% extract concentration, whereas partial inhibition was observed at 25% and 50% concentrations, which also exhibited varying effects on seed germination. The results demonstrate that the crude extract of *Aspergillus terreus* exhibits a clear concentration-dependent inhibition of *Fusarium oxysporum*, with the 100% extract showing the strongest suppression, while the 50% concentration maintains an effective balance between antifungal activity and seed viability. Recent studies confirm that *A. terreus* produces bioactive metabolites such as itaconic acid, lovastatin, and various fatty derivatives that contribute to its antifungal potential. These findings support the possible development of *A. terreus* extracts as biofungicides; however, further purification of active compounds and evaluation of phytotoxicity and field performance remain essential for agricultural application (Tedsree *et al.*, 2022 & Nagaraju *et al.*, 2025).

Conclusion

The present study demonstrated that endophytic fungi isolated from sunflower (*Helianthus annuus*) and olive (*Olea europaea*) plants possess notable antagonistic activity against several plant pathogenic fungi, including *Fusarium* sp., *Rhizoctonia solani*, and *Botrytis* sp. Among the isolates, *Phyllactinia* sp. And *Aspergillus niger* exhibited the highest inhibitory effects, indicating its strong potential as a biological control agent. In contrast, *A. terreus* showed moderate antifungal activity but displayed interesting growth-promoting effects on cucumber (*Cucumis sativus*) seeds when applied at lower extract concentrations.

The findings suggest that the bioactive compounds produced by *A. terreus* may enhance seed germination and root development at sublethal doses, although their direct antifungal efficiency against *Fusarium oxysporum* remains limited under the tested conditions. Therefore, optimizing extraction techniques or purification methods could improve the potency of these metabolites for future applications.

Overall, this research highlights the dual potential of endophytic fungi as both biocontrol agents against plant pathogens and natural growth promoters for crops. Further studies focusing on metabolite characterization, formulation, and field-level validation are recommended to advance their use as sustainable alternatives to chemical fungicides in modern agriculture.

REFERENCES

1. Adnani, M., El Hazzat, N., Msairi, S., El Alaoui, M. A., Mouden, N., Selmaoui, K., Benkirane, R., Ouazzani Touhami, A., & Douira, A. (2024). Exploring the efficacy of a *Trichoderma asperellum*-based seed treatment for controlling *Fusarium equiseti* in chickpea. *Egyptian Journal of Biological Pest Control*, 34(1), 7. <https://doi.org/10.36103/ijas.v53i3.1577>
2. Al-Nuaimy, M.A. and S. N. Hawar, (2024). Isolation and identification of endophytic fungi from *Aloe vera* leaves and chemical analysis of the alcoholic extract of the leaves using HPLC, GC, and GC-Mass devices. *Ibn Al-Haitham Journal of Pure Applied Sciences*. 34. In press
3. Anwar, Sh., F. Mahmood, N. A. Tahir, and G. F. Salih.(2022). Secondary compounds released by rhizospheric bacteria exhibit fungistatic effects against phytopathogenic fungus. *Iraqi Journal of Agricultural Sciences*, 53(5): 1174-1183. <https://doi.org/10.36103/ijas.v53i5.1631>.

4. Da Silva, S. G. M., B. A. de Melo, M. T. dos Santos, R. R. S. Rios, C. M. D. S. A. Santos, K. A. L. R. Júnior, F. C. de Maranhão, T. M. C. Santos and A. B. Fraga. 2022. Endophytic fungi: Benefits for plants and biotechnological potential. *Res. Soc. Dev.* 11(4): 27008.
5. El-Nagar, R. A., & Ghanem, A. (2024). Surface active dicationic ionic liquids as green oil spill dispersants. *Marine Pollution Bulletin*, 201, 116280.
6. Heydari, A., and M. Pessarakli. 2010. A review on biological control of fungal plant pathogens using microbial antagonists. *J. Biol. Sci.* 10(4): 273-290.
7. Jadhav, A. S., O. A. Patil, S. V. Kadam and M. A. Bhutkar. (2020). Review on Aloe Vera is used in medicinal plant. *Asian J. Pharm. Sci.* 10(1): 26-30.
8. Kharwar, R. N., A. L. Maurya, V. C. Verma, A. Kumar, S. K. Gond, S. K. and A. Mishra. (2012). Diversity and antimicrobial activity of endophytic fungal community isolated from medicinal plant *Cinnamomum camphora*. *Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.* 82(4): 557-565.
9. Mishra, V. K., A. K. Passari, P. Chandra, V. V. Leo, B. Kumar, S. Uthandi and B. P. Singh. (2017). Determination and production of antimicrobial compounds by *Aspergillus clavatonanicus* strain MJ31, an endophytic fungus from *Mirabilis jalapa* L. using UPLC-ESI-MS/MS and TD-GC-MS analysis. *PLOS One*, 12(10): e0186234.
10. Nagaraju, D., Yanjarappa, S. M., & Purushothama, C. R. A. (2025). Molecular Approaches for the Detection of Fungal Phytopathogens. CRC Press.
11. Othman, B. A. and E. S. Kakey. 2021. Pesticides bioaccumulation and their soil pollutant effect. *Iraqi Journal of Agricultural Sciences.* 52(1): 36-47.
<https://doi.org/10.36103/ijas.v52i1.1234>.
12. Sánchez, M., E. González-Burgos, I. Iglesias, and M. P. Gómez-Seranillos. (2020). Pharmacological update: properties of Aloe vera and its major active constituents. *Molecules*, 25(6):1324.

13. Segaran, G. and M. Sathivelu. (2019). Fungal endophytes: A potent biocontrol agent and a bioactive metabolites reservoir. *Biocatal. Agric. Biotechnol.* 21: 101-284.
14. Syaifudin, A. and R. S. Kasiandari. (2022). The inhibition of Fusarium wilt in Chili by Endophytic Fungi isolated from Green Betel (Piper betel L.) Leaf. *J. Nat. Sci. Math. Res.*8(2): 84-93.
15. Adnani, M., *et al.* (2024). *Trichoderma asperellum* seed treatment for controlling Fusarium equiseti. *Egyptian Journal of Biological Pest Control*, 34(1), 7.
16. El-Nagar, R. A., & Ghanem, A. (2024). Ionic liquids as green bioactive agents. *Marine Pollution Bulletin*, 201, 116280.
17. Nagaraju, D., Yanjarappa, S. M., & Purushothama, C. R. A. (2025). *Molecular Approaches for Detection of Fungal Phytopathogens*. CRC Press.