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Exploring fungal antagonists: Diversity and biocontrol potential against grapevine (*Vitis vinifera*) foliar pathogens

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ABSTRACT

Grapevine (*Vitis vinifera* L.) production is threatened by foliar pathogens like *Alternaria*, *Colletotrichum*, and *Plasmopara* leading to fungicide dependence, thereby raising concerns of residues and resistance. The present study was carried out during 2022–23 at ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Karnataka to isolate and evaluate native fungal antagonists as sustainable biocontrol options. A hemi-biotrophic oomycete *Phytophthora nicotianae* was chosen as substitute for *Plasmopara viticola*. The *in vitro* co-culture studies of the isolated 42 fungal isolates with target pathogens identified 15 isolates showing mycelia growth inhibition of pathogens by and over 35%. Molecular characterization of these isolates using ITS-1F and 4R primers highlighted the constitution of opportunistic human pathogens (*Mucor irregularis*), mushrooms (*Ganoderma* spp. and *Lentinula edodes*), phytopathogens (*Fusarium* spp. and *Pestalotiopsis* spp.) and other fungi like *Xylaria, Trichoderma, Nemania* and *Byssochlamys* spp. *Xylaria* spp. (IIHR_BCF14) demonstrated efficacy comparable to *T. harzianum* (IIHR_BCF23), inhibiting the mycelial growth of all three target pathogens. Notably, *Endomelanoconiopsis endophytica* (IIHR_BCF20), *Lntinula edodes* (IIHR_BCF26), and *Ganoderma* species (IIHR_BCF38, IIHR_BCF40 and IIHR_BCF41) showed specific antifungal activity against *A. alternata*. The study signified the complexity associated with management of *C. gloeosporioides* with very few bioagents demonstrating antifungal activity against the pathogen. The study yielded few preliminary reports on directed antagonism of few biocontrol potent fungi (*B. spectabilis, Xylaria* spp. against *P. nicotianae*; *E. endophytica* against *A. alternata*) and emphasizes on their exploitation for management of grapevine pathogens

Keywords: Biocontrol, Foliar pathogens, Fungi, Grapes, Management

Grapes (*Vitis vinifera* L.) is a global horticultural crop, with a production of 74.94 million metric tonnes (Shahbandeh 2020). It is frequently listed among the "Dirty Dozen" list by the Environmental Working Group (EWG) with highest pesticide residues that has sparked global concern. In India, grapes are the fifth most important fruit crop, earning substantial foreign returns, 417.07 million USD during 2023–24 (Economic Survey of India 2024). Its cultivation is challenged by diseases such as downy mildew, powdery mildew, anthracnose, and *Alternaria* blight, often requiring intensive fungicide use. The *Colletotrichum* genus was ranked among the top 10 fungal pathogens in 2012 due to its wide host range, destructiveness, and post-harvest losses (Dean *et al*. 2012). *Colletotrichum* and *Alternaria* persist both in field and post-harvest conditions complicating their management. *Plasmopara viticola*, a downy mildew pathogen, presents a major challenge for it being an oomycete differing from true fungi both physiologically and genetically. Its obligate nature further complicates research into alternative management strategies (Muthukumar *et al*. 2025). However, a related hemi-biotroph like *Phytophthora* may offer insights for devising effective management means through *in vitro* studies.

Over the past decade, biological control has taken greater leap in managing plant pests and diseases. The bioagents employ various mechanisms, viz. competition, predation, hyper-parasitism, production of antimicrobial metabolites and inducing plant resistance to combat phytopathogens (Zhang *et al.* 2023, Wu *et al.* 2025). Fungal antagonists specially have high reproductive rates, host specificity, and with ability to form survival structures making them sustainable and effective (Thambugala *et al*. 2020). Noteworthy examples include *Trichoderma* spp., *Ampelomyces quisqualis, Candida oleophila, Clonostachys rosea, Phlebiopsis gigantea*, and *Coniothyrium minitans*. However, more than 250 other such fungal antagonists remain underexplored. Lately, there is an increased emphasis on such novel bioagents for eco-friendly pest management.

In context of grapes, biological control research is predominantly focused on managing post-harvest pathogens (Solairaj *et al*. 2020, Chen *et al*. 2022). Considering the

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importance of foliar pathogens, the present study aimed to investigate fungi from native forests and fields for their antifungal activity against pathogens, *A. alternata*, *C. gloeosporioides* and *P. viticola* by choosing a hemi-biotrophic oomycete *P. nicotianae* as substitute for later.

MATERIALS AND METHODS

The present study was carried out during 2022–23 at ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Karnataka. The pathogens under study, *A. alternata* (PQ277028), *C. gloeosporioides* (PQ277030) and *P. nicotianae* (ON358198) were collected from the culture collection of fruit pathology laboratory (Mohankumar *et al*. 2022). Potato dextrose agar (PDA) medium (200 g potato, 20 g dextrose, 20 g agar/L) was used for revival and culture maintenance. Pathogenicity tests were conducted to verify the Koch's postulates by spraying spore suspensions from the pathogen cultures at 10^6 per mL concentration onto fresh bunches of grapes berries and leaves of betel vine (*P. nicotianae*) (Fao *et al*. 2017).

In view to expand the range of fungal bioagents against plant pathogens, macroscopic fungal structures growing in soil and on tree trunks, wooden logs, and decomposing wood was carried out from three locations: Indigenous forests in Sirsi, Uttara Kannada district, Karnataka (14.62°N, 74.85°E); local forests around Indira Gandhi Krishi Vishwavidyalay, Raipur, Chhattisgarh (21.2376°N, 81.7055°E); and fields near ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka. Of these, 14 isolates were from fields in IIHR and nearby locations, 16 from forests of Sirsi region while seven and six isolates were collected from Mushroom laboratories of Indira Gandhi Krishi Vishwavidyalaya and ICAR-Indian Institute of Horticultural Research , respectively. The fungal structures collected were isolated following the isolation technique described by Ilyas and Avin (2018) where, each fruiting body was aseptically split in half, and a small piece of clean inner tissue excised with a sterilized scalpel was inoculated onto the surface of sterilized PDA medium. The cultures developed on incubation at room temperature post 5–6 days of inoculation were purified and maintained by repeated subculturing.

Dual culture assays were performed by simultaneous culturing of the test pathogens and test biocontrol fungi for evaluation of antagonism of the bioagents (Oldenburg *et al*. 1996). A 5 mm bit cut from the periphery of fungal pathogen colony was transferred onto a PDA plate 2 cm from one edge which was then challenge inoculated with a 5 mm mycelial bit cut from the periphery of colony of test bioagents. The plates were incubated at 28±2°C and three replications were maintained per treatment while sole inoculation of pathogens served as control**.** The colony diameters of pathogens at 3-days interval up to 12 days were recorded and per cent inhibition was calculated using the following formula (Vincent 1947).

$$I = (C-T)/C \times 100$$

Where I, Inhibition per cent; C, Colony diameter of pathogen in control (cm); T, Colony diameter of the pathogen in treatment (cm). For better interpretation of the resulted inhibition of pathogens, the per cent inhibition was categorized on a scale of 1 to 4, where 1, 0–19% inhibition; 2, 20–34% inhibition; 3, 35–49% inhibition; and 4, ≥ 50% inhibition.

Biocontrol fungi that demonstrated over 35% inhibition of the target pathogens were examined for morphological interactions brought about at the periphery of the pathogen colonies. To elucidate these interactions, the fungi were classified into five categories, based on modified models from Azuddin *et al.* (2021) and Holkar *et al*. (2023) i.e. Type A, Mutual intermingling of mycelial growth in which both the fungi grow into each other at periphery; Type B, Over growth of biocontrol agent on the pathogen; Type C, Restricted growth of both the fungi on contact; Type D, Growth restriction with inhibition zone of <2 mm between the two fungi and Type E, Growth restriction with inhibition zone of >2mm between the two fungi.

The fungi showing substantial antagonism were subjected to molecular characterization. The fungi were cultured in potato dextrose broth (PDB) for a week and DNA extraction was carried by modified CTAB method following the protocol of Moller *et al*. (1992). The extracted DNA was subjected to PCR analysis using the universal fungal primers ITS-1F (5′TCC GTA GGT GAA CCT TGC GG 3′) and ITS-4R (5′TCC TCC GCT TAT TGA TAT GC 3′) for amplification of 5.8S rDNA region (White *et al*. 1990). The obtained sequences were aligned using Bio Edit Sequence Alignment Editor Version 7.0.5 software to obtain consensus sequences and compared with previous repositories in NCBI data base (http://blast.ncbi.nlm.nih.gov/) using BLAST. The organisms were identified based on per cent similarity.

The experiments were repeated twice and the pooled per cent inhibition data from the replications was analysed statistically in OPSTAT software developed by O P Sheoran, Professor Statistics at COBS & HCCS, Haryana Agricultural University, Hisar, Haryana in completely randomized design (CRD), separately for each pathogen using analysis of variance technique at 1% probability. The data were arcsine transformed to attain normal distribution and stabilize variances.

RESULTS AND DISCUSSION

The pathogenicity tests revealed the authenticity of the pathogen cultures to the previous workers (Simmons 2007, Mohankumar *et al*. 2022) as well as to the original key descriptions in terms of symptom development as well as morphological characteristics verifying the Koch's postulates.

The dual culture studies of the test bioagents with *A. Alternata* for 12 days post-inoculation showed five fungal strains IIHR_BCF18, IIHR_BCF14, IIHR_BCF23, IIHR_BCF26, and IIHR_BCF20 with over 50% inhibition in mycelial growth of the pathogen (Score-4). Among these, IIHR_BCF23 demonstrated the highest efficacy, with a

remarkable 68.6% inhibition. Additionally, six fungal strains IIHR_BCF01, IIHR_BCF17, IIHR_BCF19, IIHR_BCF38, IIHR_BCF40, and IIHR_BCF41 also achieved a significant level of inhibition (Score-3), with inhibition percentage ranging from 35–50%. Overall, 25% of the tested fungi exhibited substantial antagonistic activity against *A. alternata*, with five strains showing particularly significant potential for further development and application (Table 1). Previous workers Daroodi *et al*. (2022) and Zhang *et al*. (2024) similarly explored and reported the biocontrol potential of endophytic fungi, *Acrophialophora jodhpurensis* and *Talaromyces muroii* SD1-4 against *A. alternata.*

With respect to *C. gloeosporioides*, two fungal strains, IIHR_BCF14 and IIHR_BCF23, exhibited significant inhibition, with inhibition rates of 57.7 and 56.2%, respectively, both classified under Score-4. Additionally, three fungi IIHR_BCF18, IIHR_BCF17, and IIHR_BCF22 demonstrated significant inhibition within the range of 35–50% (Score-3) (Table 1). The study highlighted the challenges in managing *C. gloeosporioides*, a pathogen known for its destructive nature with majority of biocontrol fungi tested falling into score-1 and score-2. Management of *C. gloeosporioides* by fungal antagonists has long been actively investigated. *Trichoderma* were reported potentially inhibitory to the pathogen (Freeman *et al*. 2004). Lately, fungal endophytes have been emphasized and extensively studied to devise management means against the pathogen (Bian *et al*. 2021, Sharma *et al*. 2021, Holkar *et al*. 2023).

Effective management of *P. nicotianae,* the chosen substitute to the obligate pathogen *P. viticola* has great importance due to the inherent difficulties associated with oomycete pathogens (Volynchikova and Kim 2022). We have identified six fungi, IIHR_BCF18, IIHR_BCF14, IIHR_BCF17, IIHR_BCF23, IIHR_BCF21 and IIHR_BCF35 demonstrating substantial mycelial growth inhibition of *P. nicotianae* exceeding 50% (score-4). The maximum inhibition of the pathogen was encountered on co-culturing with fungi, IIHR_BCF14, and IIHR_BCF18 with 59.6 and 59.2%, respectively. There were only three fungi grouped under score-3 with inhibition per cent ranging from 35–49 i.e. IIHR_BCF01, IIHR_BCF29 and IIHR_BCF15 (Table 1). Similar means of management of *P. nicotianae* mediated by fungal antagonists was attempted by earlier workers (Liu *et al*. 2022, Volynchikova and Kim 2022).

Among the isolated fungi, approximately 75% exhibited inhibition percentage below 35%, regardless of the test pathogen. Notably, the isolates IIHR_BCF14 and IIHR_BCF23 were found to be highly effective against all three test pathogens, each achieving over 50% reduction in mycelial growth. Additionally, IIHR_BCF18 and IIHR_BCF17 achieved approximately 40% or greater inhibition across all three pathogens. The co-culture studies analysed for different interactions revealed Type B interaction to be most prevalent characterized by the overgrowth of the biocontrol agent on the target pathogen. The second most common interaction observed was Type C shown by five isolates, IIHR_BCF01, IIHR_BCF17, IIHR_BCF26, IIHR_BCF29 and IIHR_BCF35 which involved mutual restriction of growth upon contact between the biocontrol fungi and the pathogen. Type E interaction was identified in three isolates, where pathogen growth was impeded with inhibition zones exceeding 2 mm (Table 2). Previous studies by Azuddin *et al*. (2021) and Holkar *et al.* (2023) similarly explored biocontrol interactions of endophytic fungi with phytopathogens. The observed inhibition zone was suggested to be likely the result of interaction mediated by metabolites and would be desirable for exploitation of biopesticides

Pigmentation along the periphery of pathogen colonies was also observed in co-culture studies with *A. alternata* and *C. gloeosporioides*. *A. alternata* exhibited peripheral pigmentation when co-cultured with the biocontrol fungi, IIHR_BCF04, IIHR_BCF18, IIHR_BCF23, IIHR_BCF32, IIHR_BCF28, IIHR_BCF24, IIHR_BCF17, and IIHR_BCF14. Similarly, *C. gloeosporioides* showed peripheral pigmentation in the presence of biocontrol fungi, IIHR_BCF08, IIHR_BCF18, IIHR_BCF22, IIHR_BCF14, IIHR_BCF28, IIHR_BCF20, and IIHR_BCF26. Three fungi that rendered pigmentation along the periphery of both the pathogens were IIHR_BCF14, IIHR_BCF18 and IIHR_BCF22. Microscopic examination of the hyphae at the periphery revealed morphological alterations such as hyphal swellings and distortions. These changes were consistent with findings reported in the literature, which attribute such deformations to protoplasmic dissolution and disintegration caused by metabolic stress from fungal bioagents (Rahman *et al*. 2007).

The fungi showing substantial antagonism of test pathogens by and over 35% were subjected to molecular characterization. This included 11, 5 and 9 fungi against *A. alternata*, *C. gloeosporioides* and *P. nicotiane*, respectively all together accounting for 15 fungal isolates. Based on BLAST search, the isolates were identified into 9 genera and 10 species with percentage of similarity from 97–100% (Table 3). The group presented a great variability constituting well established bioagents, opportunistic human pathogens, mushrooms and certain other novel fungi. The fungal isolates, IIHR_BCF18 and IIHR_BCF22 identified as *Mucor irregularis* despite showing substantial inhibitory effects against the test pathogens were excluded for further studies for it being an opportunistic human pathogen.

Molecular characterization of the isolates, IIHR_BCF14 and IIHR_BCF23 showing significant antagonism with over 50% inhibition of mycelial growth against all three test pathogens, presented them as *Xylaria* spp. and *T. harzianum*, respectively. Few fungi encountered, *M. irregularis, T. harzianum, P. microspora* and *F. proliferatum* were assumed to be co-inhabitants or parasites of the picked macroscopic fungal structures. Fungi such as *Xylaria* spp. and *T. harzianum* could be promising candidates for further evaluation in the management of *C. gloeosporioides*. This potential is supported by earlier studies (Soytong *et al*. 2005, Luciana *et al.* 2018), which suggest these fungi may offer effective biocontrol options. The antagonism of *Xylaria* spp. against *Alternaria, Colletotrichum* and *Phytophthora*

Table 1 *In vitro* antifungal activity of various fungal antagonists against *A. alternata*, *C. gloeosporioides* and *P. nicotianae*

Test biocontrol fungus	% inhibition of mycelial growth		
	A. alternata	*C. gloeosporioides*	*P. nicotianae*
IIHR_BCF01	45.4 (42.4) ± 0.9	21.7 (27.8) ± 0.7	48.1 (44.0) ± 1.2
IIHR_BCF03	26.3 (30.9) ± 1.1	34.5 (35.9) ± 0.9	24.2 (29.5) ± 0.9
IIHR_BCF04	26.7 (31.1) ± 2.3	17.6 (24.8) ± 0.9	26.1 (30.7) ± 0.8
IIHR_BCF05	17.9 (24.8) ± 4.4	19.9 (26.5) ± 0.2	21.6 (27.7) ± 1.0
IIHR_BCF06	17.9 (25.1) ± 1.9	26.2 (30.8) ± 1.1	24.6 (29.8) ± 0.6
IIHR_BCF07	19.8 (26.4) ± 2.7	33.3 (35.3) ± 0.0	23.9 (29.2) ± 1.1
IIHR_BCF08	20.9 (27.2) ± 2.5	14.9 (22.7) ± 1.2	17.4 (24.7) ± 1.3
IIHR_BCF09	11.8 (20.1) ± 1.2	16.9 (24.2) ± 0.7	24.3 (29.5) ± 0.7
IIHR_BCF18	58.6 (49.9) ± 0.2	44.2 (41.7) ± 0.6	59.2 (50.3) ± 0.6
IIHR_BCF34	32.9 (35.0) ± 0.3	30.3 (33.4) ± 2.4	19.6 (26.3) ± 1.9
IIHR_BCF43	21.8 (27.9) ± 0.5	22.4 (28.3) ± 2.1	18.1 (25.2) ± 0.7
IIHR_BCF14	60.2 (50.9) ± 1.6	57.7 (49.4) ± 0.1	59.6 (50.6) ± 0.7
IIHR_BCF10	15.3 (23.0) ± 0.3	20.6 (26.9) ± 3.2	21.1 (27.3) ± 1.9
IIHR_BCF24	23.8 (29.2) ± 0.7	22.8 (28.5) ± 1.1	23.8 (29.2) ± 0.6
IIHR_BCF32	15.7 (23.3) ± 1.7	19.5 (26.2) ± 0.5	20.4 (26.8) ± 1.3
IIHR_BCF17	39.8 (39.1) ± 1.2	44.9 (42.1) ± 1.3	53.9 (47.3) ± 0.5
IIHR_BCF19	16.5 (23.9) ± 1.7	29.2 (32.7) ± 1.4	24.5 (29.7) ± 0.7
IIHR_BCF28	24.1 (29.4) ± 1.2	20.6 (26.9) ± 1.2	17.4 (24.6) ± 0.3
IIHR_BCF33	29.5 (32.9) ± 0.7	11.2 (19.5) ± 2.0	18.8 (25.7) ± 2.5
IIHR_BCF23	68.6 (55.9) ± 1.2	56.2 (48.6) ± 0.5	58.1 (49.7) ± 0.4
IIHR_BCF27	17.9 (25.1) ± 1.9	19.8 (26.4) ± 2.0	22.2 (28.1) ± 2.0
IIHR_BCF22	25.6 (30.4) ± 1.0	43.6 (41.2) ± 0.9	13.9 (21.8) ± 3.0
IIHR_BCF31	29.2 (32.7) ± 1.6	21.3 (27.5) ± 1.6	21.1 (27.3) ± 0.8
IIHR_BCF42	21.9 (27.9) ± 1.4	31.8 (34.3) ± 1.1	14.7 (22.5) ± 2.3
IIHR_BCF26	53.1 (46.8) ± 1.2	23.2 (28.8) ± 1.1	24.5 (29.7) ± 0.6
IIHR_BCF16	12.3 (20.5) ± 1.9	22.4 (28.3) ± 2.1	12.0 (20.2) ± 2.8
IIHR_BCF11	13.9 (21.8) ± 0.8	19.5 (26.2) ± 1.6	19.9 (26.5) ± 1.7
IIHR_BCF13	23.8 (29.2) ± 0.2	16.4 (23.8) ± 2.7	14.7 (22.4) ± 2.5
IIHR_BCF12	29.2 (32.7) ± 1.6	18.3 (25.3) ± 2.4	19.2 (26.0) ± 0.8
IIHR_BCF30	32.3 (34.6) ± 1.3	22.5 (28.3) ± 0.6	19.6 (26.3) ± 1.9
IIHR_BCF20	55.0 (47.9) ± 0.3	6.4 (14.5) ± 1.8	16.6 (24.1) ± 0.5
IIHR_BCF21	29.4 (32.8) ± 1.2	12.3 (20.5) ± 0.1	51.4 (45.8) ± 2.0
IIHR_BCF35	21.1 (27.3) ± 1.2	10.2 (18.7) ± 0.5	50.9 (45.5) ± 1.1
IIHR_BCF29	5.5 (13.6) ± 0.1	15.6 (23.2) ± 1.1	46.9 (43.2) ± 0.8
IIHR_BCF15	5.5 (13.6) ± 0.1	12.2 (20.5) ± 1.9	44.1 (41.6) ± 1.8
IIHR_BCF25	23.4 (28.9) ± 2.1	27.9 (31.9) ± 1.0	9.0 (17.5) ± 0.6
IIHR_BCF36	27.3 (31.5) ± 3.0	6.4 (14.6) ± 1.0	15.1 (22.8) ± 1.4
IIHR_BCF37	21.4 (27.5) ± 3.5	13.6 (21.6) ± 2.9	33.6 (35.4) ± 1.8
IIHR_BCF38	43.7 (41.4) ± 1.1	11.9 (20.2) ± 1.0	31.9 (34.4) ± 2.1
IIHR_BCF39	27.3 (31.5) ± 1.3	8.9 (17.4) ± 0.9	23.3 (28.8) ± 1.9
IIHR_BCF40	43.3 (41.1) ± 0.7	18.8 (25.7) ± 0.9	15.9 (23.5) ± 1.5
IIHR_BCF41	41.2 (39.9) ± 0.1	8.5 (16.8) ± 2.3	25.4 (30.3) ± 1.3
CD (p=0.01)	3.041	3.119	2.896
SEM±	0.81	0.84	0.78
Coefficient of Variation	4.473	5.212	4.343

*The % inhibition data of each of the pathogens was analysed separately through completely randomized design; figures in parentheses are arc-sine transformed values, alongside provided are values of standard deviation, and each tabulated value of per cent inhibition is the mean of three replications.

Table 2 Mycelial interaction types of bioagents with target pathogens at interface on co-culturing

Test fungus	Interface interaction type of pathogen and biocontrol agent		
	A. alternata	*C. gloeosporioides*	*P. nicotianae*
IIHR_BCF01	C	D	B
IIHR_BCF14	B	B	B
IIHR_BCF15	A	A	B
IIHR_BCF17	B	C	B
IIHR_BCF18	B	B	B
IIHR_BCF20	C	C	C
IIHR_BCF21	D	B	B
IIHR_BCF22	B	B	B
IIHR_BCF23	B	B	B
IIHR_BCF26	D	B	C
IIHR_BCF29	C	A	B
IIHR_BCF35	C	C	B
IIHR_BCF38	B	B	B
IIHR_BCF40	B	B	B
IIHR_BCF41	B	B	B

Type A, Mutual intermingling of mycelial growth in which both the fungi grow into each other at periphery; Type B, Over-growth of biocontrol agent on the pathogen; Type C, Restricted growth of both the fungi on contact; Type D, Growth restriction with inhibition zone of >2 mm between the two fungi.

was evident from the earlier reports of Sánchez-Ortiz *et al.* (2016) and Wang *et al*. (2022).

Molecular characterization of the fungal isolates showing potential inhibitory effects against *P. nicotianae* were identified as *B. spectabilis*, *T. harzianum*, *Xylaria* spp., *M. irregularis*, and *P. microspora*. These fungi have been earlier reported with antagonism against various phytopathogens (Rodrigo *et al*. 2017, Brooks *et al*. 2022, Atamanchuk *et al*. 2024). Except for *T. harzianum* our work is among the foremost reports suggesting the specific antagonism of these fungi against *P. nicotiane*.

Interesting revelations are of the fungal isolates identified as *Endomelanoconiopsis endophytica* and *Byssochlamys spectabilis* with notable inhibition of *A. alternata* and *P. nicotiane*, respectively. The shiitake mushroom, *L. edodes* also finds its place with antagonistic effects against *A. alternata* and *P. nicotiane*. Remarkably, *P. microspora* was found to inhibit the mycelial growth of *P. nicotianae* by over 50%, while *F. proliferatum* showed antagonism against both *A. alternata* and *P. nicotianae*. Despite their inhibitory effects, these fungi are plant pathogens themselves and thus are not suitable for biocontrol. However, they may be valuable as sources of metabolites with antimicrobial potential.

The effectiveness of bioagents varies based on their mechanisms of action, which influences their specificity towards different pathogens. Such pathogen specificity was encountered with respect to *E. endophytica* (IIHR_BCF20), *L. edodes* (IIHR_BCF26), *G. Sichuanense* (IIHR_BCF40 and IIHR_BCF41) and *G. lucidum* (IIHR_BCF38) against *A. alternata* with no notable effects against the other pathogens. Although previous studies have highlighted the antagonistic properties of *E. endophytica* against plant pathogens (Azuddin *et al*. 2021), our research provides preliminary documentation of its directed antagonism against *A. alternata*.

Three mushroom isolates identified as *Ganoderma*

Table 3 Molecular characterization of the identified potential fungal antagonists and their range of antifungal activity against the target pathogens

Fungal isolate	Species identification	NCBI accession number	Antagonistic against		
			A. alternata	*C. gloeosporioides*	*P. nicotianae*
IIHR_BCF01	*Fusarium proliferatum*	PQ276953	*		*
IIHR_BCF14	*Xylaria* spp.	PQ276976	**	**	**
IIHR_BCF15	*Xylaria* spp.	PQ277034			*
IIHR_BCF17	*Byssochlamys spectabilis*	PQ276992	*	*	**
IIHR_BCF18	*Mucor irregularis*	PQ276997	**	*	**
IIHR_BCF20	*Endomelanoconiopsis endophytica*	PQ276998	**		
IIHR_BCF21	*Xylaria* spp.	PQ276999			**
IIHR_BCF22	*Mucor irregularis*	PQ277019		*	
IIHR_BCF23	*Trichoderma harzianum*	PQ277020	**	**	**
IIHR_BCF26	*Lentinula edodes*	PQ283242	**		
IIHR_BCF29	*Lentinula edodes*	PQ284143			*
IIHR_BCF35	*Pestalotiopsis microspora*	PQ277027			**
IIHR_BCF38	*Ganoderma lucidum*	PQ277033	*		
IIHR_BCF40	*Ganoderma sichuanense*	PQ277031	*		
IIHR_BCF41	*Ganoderma sichuanense*	PQ277032	*		

**, Mycelial growth inhibition range above 50%; *, Mycelial growth inhibition range of 35–49%.

Table 4 Test biocontrol fungi rendering peripheral pigmentation of pathogens and their molecular information

Fungal Bioagent	Molecular identification	NCBI accession number	Peripheral pigmentation shown by pathogen
IIHR_BCF04	*Bolbitius* spp.	PQ282301	*A. alternata*
IIHR_BCF23	*Trichoderma harzianum*	PQ277020	*A. alternata*
IIHR_BCF32	*Lentinula edodes*	PQ286551	*A. alternata*
IIHR_BCF24	*Nemania primolutea*	PQ277021	*A. alternata*
IIHR_BCF17	*Byssochlamys spectabilis*	PQ276992	*A. alternata*
IIHR_BCF18	*Mucor irregularis*	PQ276997	*A. alternata* and *C. gloeosporioides*
IIHR_BCF28	*Aspergillus* spp.	PQ285280	*A. alternata* and *C. gloeosporioides*
IIHR_BCF14	*Xylaria* spp.	PQ276976	*A. alternata* and *C. gloeosporioides*
IIHR_BCF08	*Agrocybe pediades*	PQ276974	*C. gloeosporioides*
IIHR_BCF22	*Mucor irregularis*	PQ277019	*C. gloeosporioides*
IIHR_BCF20	*Endomelanoconiopsis endophytica*	PQ276998	*C. gloeosporioides*
IIHR_BCF26	*Lentinula edodes*	PQ283242	*C. gloeosporioides*

lucidum and *Ganoderma sichuanense* demonstrated antifungal activity exclusively against *A. alternata*, with inhibition percentages ranging from 35–49%. The encountered antifungal activity of the mushrooms, viz. *Ganoderma* spp. and *L. edodes* against phytopathogens was also evident from the previous studies (Godeanu-Matei *et al*. 2016, Kang *et al*. 2017, Ragupathi *et al*. 2021). Such potentiality of antifungal activity of various biocontrol fungi like *Trichoderma, Lentinus, Pleurotus, Leucopaxillus* against *A. alternata* was documented by earlier workers (Rajput *et al* 2013, Feleke and Doshi 2017).

Noteworthy mentions with respect to rendered pigmentation were of three fungi, *M. irregularis* and *Aspergillus* spp. as well as *Xylaria* spp. that showed pigmentation in both the pathogens. Other interesting fungi from the study were *Nemania primolutea*, *Agrocybe pediades*, and *Bolbitius* spp. with the latter two being mushrooms stood out for their unique interactions. The molecular data of the test bioagents showing peripheral pigmentation of the respective pathogens is presented in Table 4.

Overall, these findings contribute to the growing body of research on fungal bioagents for sustainable crop management, with promising avenues for future field trials and metabolite-based biopesticide development.

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