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RESEARCH

Characterization of rhizospheric fungi and their in vitro antagonistic potential against myco-phytopathogens invading *Macrotyloma uniflorum* plants

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Abstract
Microorganisms have become more resistant to pesticides, which increases their ability to invade and infect crops resulting in decreased crop productivity. The rhizosphere plays a crucial role in protecting plants from harmful invaders. The purpose of the study was to investigate the antagonistic efficiency of indigenous rhizospheric fungal isolates against phytopathogens of *M. uniflorum* plants so that they could be further used as potent Biocontrol agents. Thirty rhizospheric fungal isolates were collected from the roots of the *Macrotyloma uniflorum* plant and initially described morphologically for the present study. Further, in vitro tests were conducted to evaluate the antifungal activity of these strains against four myco-phytopathogens namely *Macrophamina phaseolina, Phomopsis* sp. PhSFX-1, *Nigrospora oryzae,* and *Boeremia exigua*. These pathogens are known to infect the same crop plant, *M. uniflorum*, and cause declines in crop productivity. Fifteen fungal strains out of the thirty fungal isolates showed some partial antagonistic activity against the myco-phytopathogens. The potent fungal isolates were further identified using molecular techniques, specifically based on the internal transcribed spacer (ITS) region sequencing. *Penicillium mallochii, Cladosporium pseudocladosporioides, Aspergillus chevalieri, Epicoccum nigrum, Metarhizium anisopliae*, and *Mucor irregularis* were among the strains that were identified. These potent fungal strains showed effective antagonistic activity against harmful phytopathogens. Current findings suggest that these strains may be taken into consideration as synthetic fungicides which are frequently employed to manage plant diseases alternatives.

Keywords Antagonist activity · Bio-control agents · Phytopathogens · Rhizosphere fungi

Introduction

Horse gram (*Macrotyloma uniflorum*) is an underutilized and unexplored crop plant belonging to Leguminaceae family. This crop is reported to be resistant to many abiotic stresses like heavy metal stress and drought stress. This crop is used to treat kidney stones, edema, menstrual pains, piles, renal stones, healing wounds, and many more medicinal purposes (Rawat et al. 2023a, 2023b). Microorganisms are present in almost every habitat of our planet including extreme hot and cold environments. They are responsible for various functions inside the living body and also help in recycling minerals as decomposers. They live together with mixed communities and form a complex microenvironment termed a microbiome (Li et al. 2021). The microbiome includes different microbial communities coexisting in a particular environment. The rhizosphere microbiome is responsible for plant growth and health and somehow the microbes residing around the roots of plants help them to grow properly by avoiding the invasion of harmful pathogens inside plant roots either by secreting some chemicals, siderophores, etc. (Rawat et al. 2020). A plant's rhizosphere, or root system, affects its resistivity

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because it draws in beneficial bacteria and drives off undesirable ones. The myco-phytopathogens, like *Macrophamina phaseolina, Nigrospora oryzae, Boeremia exigua, Phomopsis* sp. PhSFX-1, are the most common phytopathogens infecting not only this legume plant but also commercially important crop plants like tomato, pepper, chickpea, mungbean, maize, and rice (Wang et al. 2017; Udayanga et al. 2011; Lan and Duan 2022). These pathogens affect the quality and quantity of crops resulting in a decline in production (Banaras et al. 2020; Khan and Javaid 2020). Synthetic pesticides are used for attaining a high yield of production but they come with greater risk to human health and the environment.

Fungi are the second largest group after insects and the key component of tropical ecosystems throughout the world and intimately associated with crucial processes like the decomposition, recycling, and transportation of nutrients in different environments (Chander 2016; Hawksworth and Lücking 2017; Wu et al. 2019). Fungi are one of the most diverse groups of Eukarya and represent an important functional component of the soil microbial communities (Tan et al. 2017), which constitute more of the soil biomass than bacteria, depending on soil depth and nutritional conditions (Paulina et al. 2016). Soil fungi are known to play an important role in decomposition via soil nutrient recycling and accumulation of soil organic matter and in plant health and development (Bridge and Spooner 2001; Martin et al. 2000). Various studies reported the following plant growth-promoting fungi genera *Gliocladium*, *Penicillium*, *Aspergillus*, *Phoma*, *Phytophthora*, *Rhizoctonia*, *Talaromyces*, *Trichoderma* are used to improve tomato, orange, apple, pear, cucumber, carrot, and other plants' growth and further promote the plants' innate immunity and the production of various necessary secondary metabolites by the plants (Khan et al. 2021, Khan and Javaid 2022a, 2022b; Rawat et al. 2023a, 2023b; Attia et al. 2022; Kuzin et al. 2020; Cantabella et al. 2020). Plant growth-promoting fungi (PGPF) perform the following functions in plants: antagonistic or biocontrol potential by competing for space and nutrients, growth hormone production (Akinola and Babalola 2021), mineral solubilization, mycoparasitic and saprophytic resistance, root colonization, and induced systemic resistance (ISR) in plants (Shasmita et al. 2022). Aside from the above roles mentioned, PGPF suppress the invasion of phytopathogens on tomato plants as well as other crop plants, they contribute to the improvement of nutrients in the soil, and they produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase and other phytohormones to reduce the production of ethylene in the plants (Adedayo et al. 2022). The most common fungi used as potent antagonist are genus *Trichoderma* but it also has some limitations. The present study is designed to isolate, characterize and identify the useful rhizosphere fungi and to test their antagonist potential against harmful pathogens and their effects on plant growth promotion. This will help in exploring more fungi against soil-borne pathogens and biopesticide development. Further, the active metabolites of potent fungi could be identified through metabolomic studies for biological activities like antifungal and anti-cancer.

Materials and methods

Isolation of myco-phytopathogens

The *M. uniflorum* plants were planted and collected in Bhimtal town of Nainital, Uttarakhand, India, during the 2019–2020 growing season, from September to November. The stem, pod, and leaf samples of *M. uniflorum* plants expressing symptoms of rot disease were collected in sterile bags. The samples were surface sterilized using 0.1% sodium hypochlorite for 3 min followed by three consecutive washings with sterilized distilled water (SDW). The samples were chopped into small pieces and inoculated in Potato Dextrose Agar media (PDA) for fungus isolation. All the PDA Plates were incubated at 28 ± 2 °C for 7 days.

Pathogenicity assay

Koch's Postulates method was used for pathogenicity assay (Ross and Woodward 2016). The test was conducted on healthy plants at their early stage by injecting fungal spore suspension. The soil used for seed sowing was autoclaved thrice to kill native microorganisms. The pots were placed inside the greenhouse under controlled environment at 25 ± 2 °C up to 20 days.

Isolation of rhizosphere fungal strains

The *M. uniflorum* seeds of resistant varieties VG-8 and VG-19 were obtained from ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand (33.5651° N, 73.0169° E) known to be resistant for many fungal rot diseases including Anthracnose. Rhizospheric soil samples along with healthy roots were collected from the *M. uniflorum* plants in mid-period and 10 days before harvesting plants.

Antagonistic assay against myco-phytopathogens

Antagonism assay was performed to check the potential of rhizospheric fungal strains in mycelial growth inhibition against *Macrophamina phaseolina, Phomopsis* sp. PhSFX-1, *Nigrospora oryzae*, and *Boeremia exigua* in vitro. A seven-day-old culture on potato dextrose agar (PDA) was used in this experiment. Briefly, a 5 mm Rhizospheric fungal mycelial disc was kept on one side of PDA plates and pathogenic cultures were kept on the other side of the fungal plugs at a 2 cm distance. Controls consisted of single cultures of the tested pathogen strains. Fungal antagonism was tested in triplicate and plates were incubated at 25 ± 2 °C for about 7 days. The antagonistic potential was evaluated as inhibition of the mycelial radial growth of pathogens against each Rhizospheric fungal strain, where *R* and *r* are the radii of fungal mycelial growth in control and treatment, respectively.

Calculation

The antagonistic index was accessed according to the following formula:

Antagonistic Index:

$$\frac{RM - rm \times 100}{RM}$$

RM: radius of the pathogen in the control plate.
rm: radius of the pathogen in the dual culture plate.

Characterization of myco-phytopathogens and rhizosphere fungal strains

Microscopic analysis

Initially, the pathogens and Rhizosphere fungal strains were identified based on morphological characteristics. The shape, color, and texture of fungal isolate were observed visually on PDA plates. The sporangial shape and size of each isolate were observed at 200 × magnification under a compound microscope. Further, the pathogenic strains confirmed in pathogenicity assays and potent antagonist Rhizosphere fungal strains were then subjected to molecular-based identification.

Molecular characterization

The identification of isolates was carried out at the sequencing facility of the National Centre for Microbial Resource (NCMR), National Centre for Cell Science,

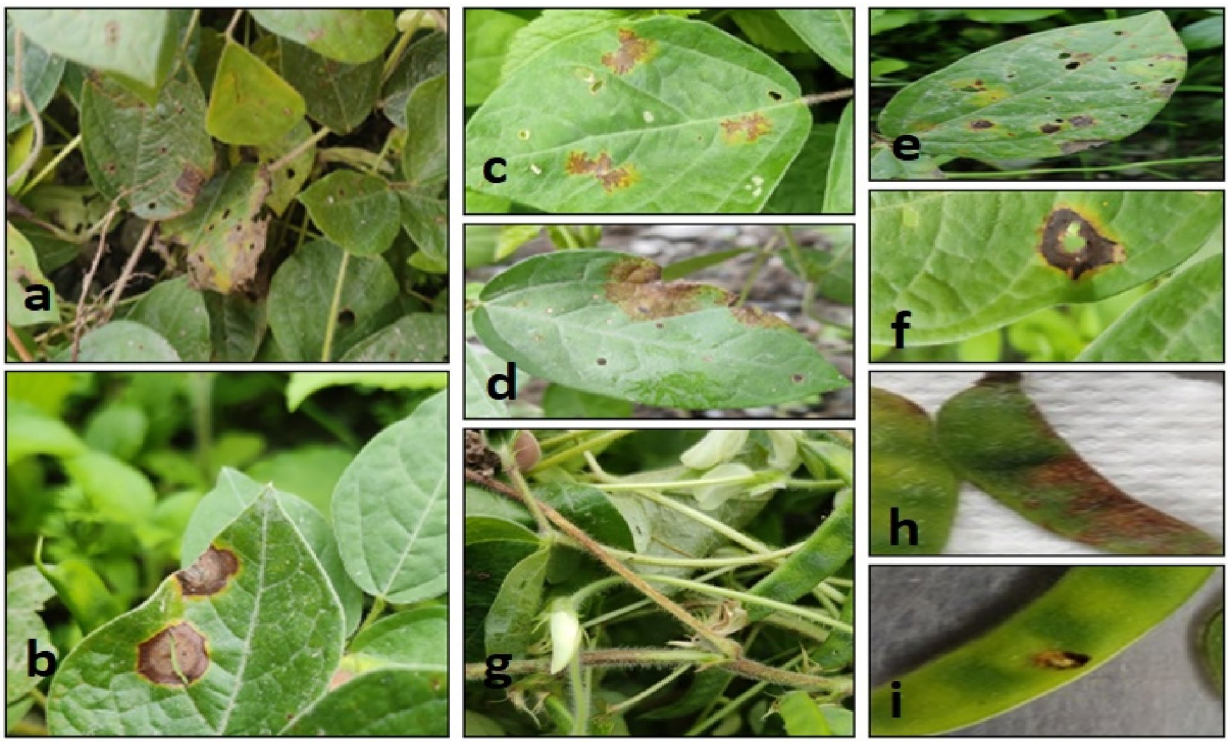


Fig. 1 Rot lesion symptoms on leaves, seed pods and stems of *Macrotyloma uniflorum* plants grown in the field. **a**–**f** Symptoms of rot on leaves. **g** Symptoms on the stem. **h, i** Symptoms on seed pods

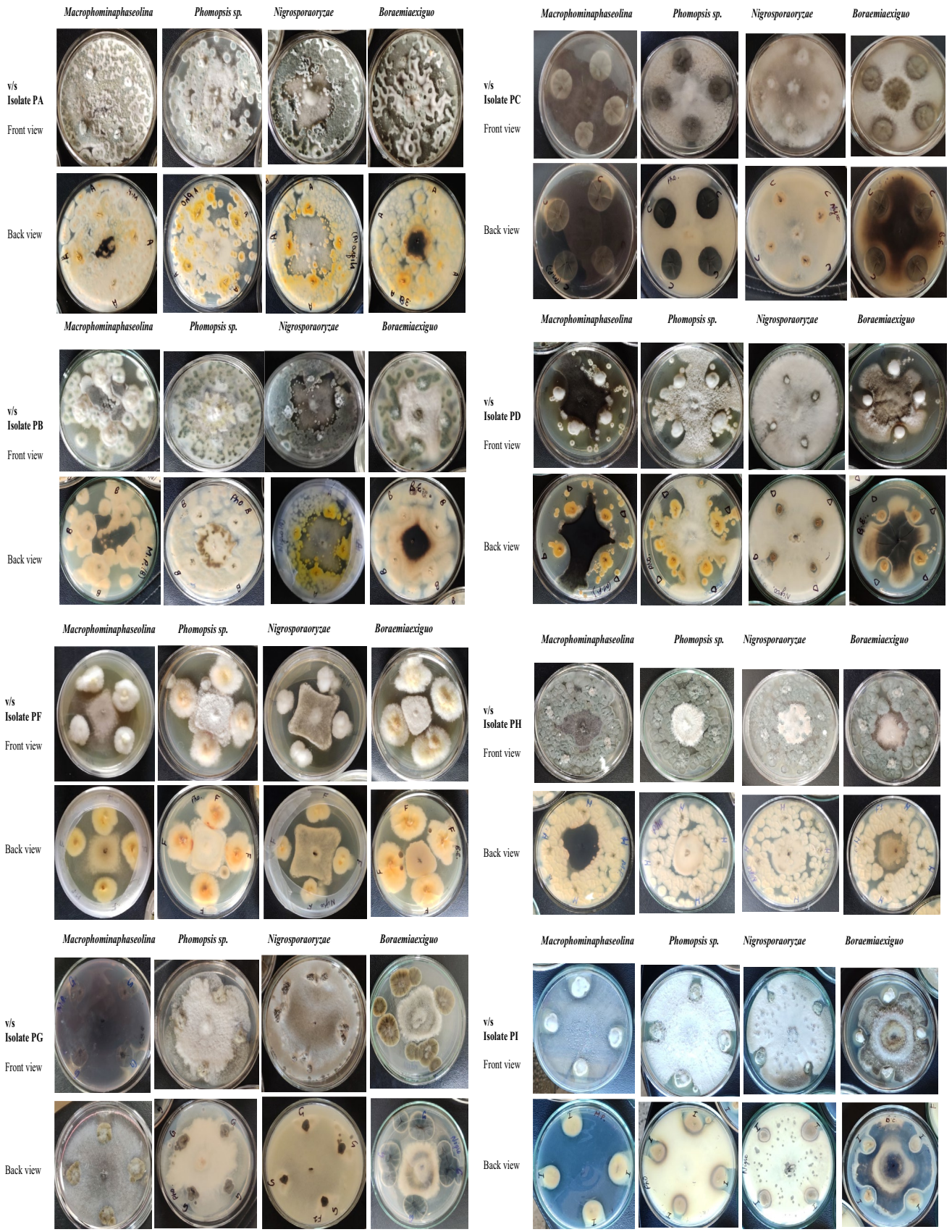


Fig. 2 Rizospheric fungal isolates showing antagonistic activity against phytopathogens *Macrophamina phaseolina, Phomopsis* sp. PhSFX-1**,** *Nigrospora oryzae*, and *Boeremia exigua*, respectively

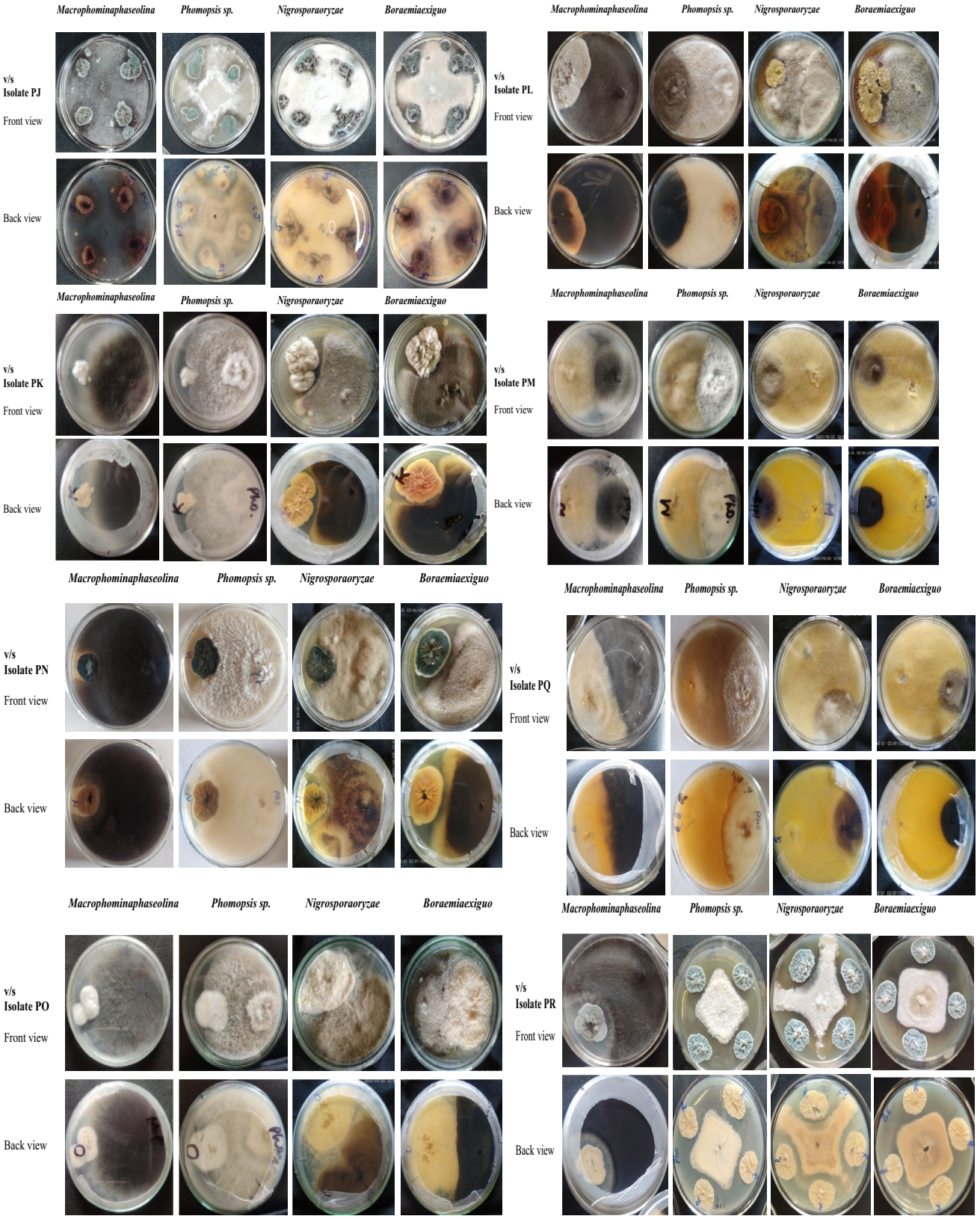


Fig. 2 (continued)

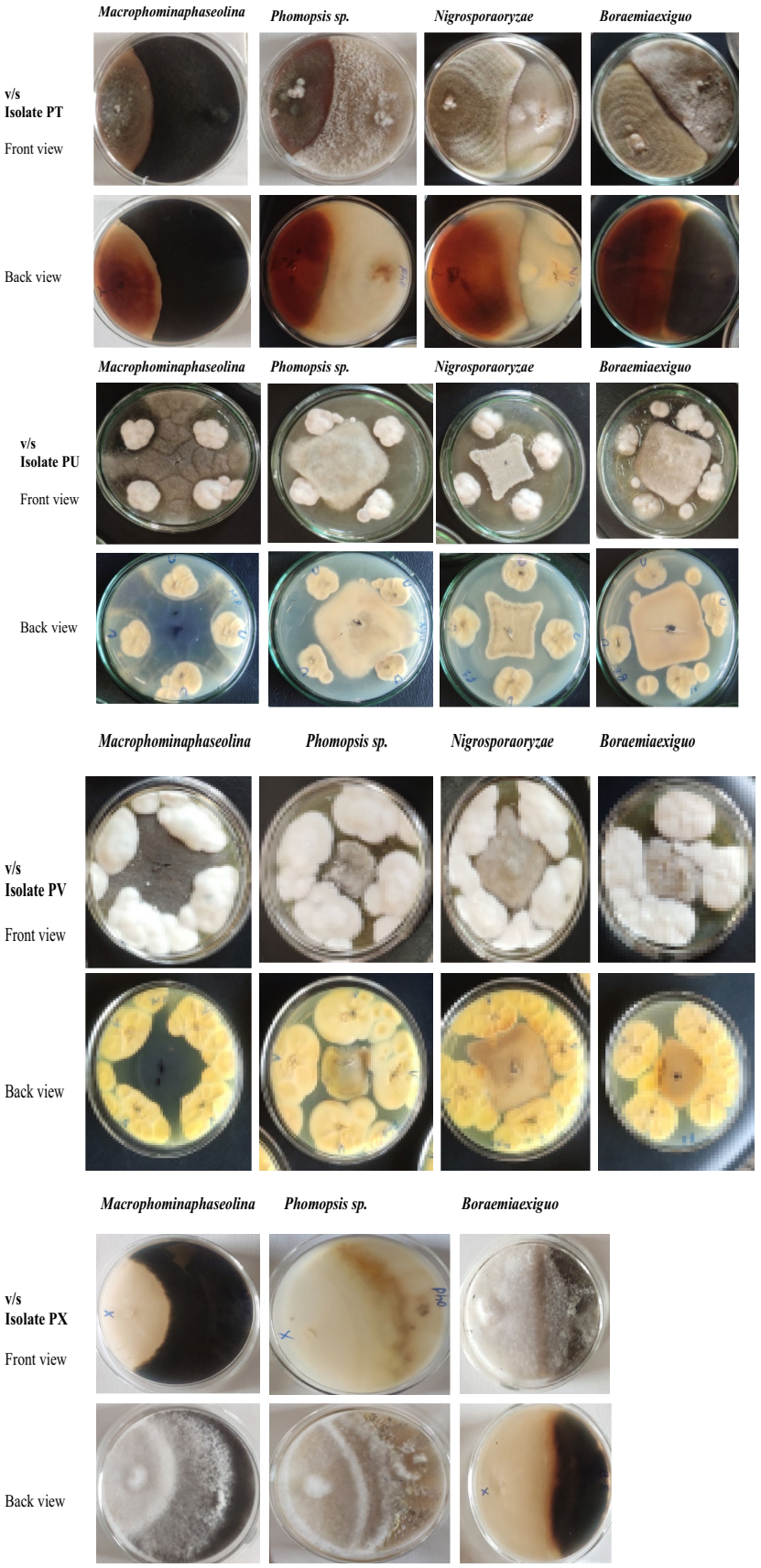


Fig. 2 (continued)

Table 1 Antagonistic Index % of isolates against myco-phytopathogens and the type of inhibition (where ***(a)***—locked at point of contact, ***(b)***—intermingled, ***(c)***—overlapping, ***(d)***—clear zone between isolate and pathogen)

S. no	Fungal isolates	Antagonistic Index %			
		Macrophomina phaseolina (type of inhibition)	*Nigrospora oryzae* (type of inhibition)	*Boeremia exigua* (type of inhibition)	*Phomopsis* sp. PhSFX-1 (type of inhibition)
1.	PA	50% ***(a)***	54% ***(b)***	28% ***(b)***	52% ***(a)***
2.	PB	45% ***(b)***	50% ***(b)***	45% ***(d)***	30% ***(a)***
3.	PC	47% ***(b)***	23% ***(a)***	46% ***(a)***	32% ***(b)***
4.	PD	39% ***(d)***	30% ***(d)***	20% ***(a)***	32% ***(d)***
5.	PF	40% ***(d)***	35% ***(d)***	60% ***(d)***	50% ***(d)***
6.	PG	30% ***(a)***	59% ***(b)***	51% ***(b)***	23% ***(b)***
7.	PH	50% ***(d)***	45% ***(b)***	55% ***(b)***	60% ***(d)***
8.	PI	29% ***(d)***	25% ***(b)***	37% ***(a)***	28% ***(d)***
9.	PJ	37% ***(a)***	45% ***(b)***	56% ***(b)***	30% ***(a)***
10.	PK	30% ***(a)***	40% ***(d)***	43% ***(d)***	28% ***(b)***
11.	PL	40% ***(b)***	30% ***(a)***	46% ***(a)***	49% ***(d)***
12.	PM	55% ***(b)***	35% ***(b)***	30% ***(b)***	57% ***(a)***
13.	PN	30% ***(b)***	35% ***(a)***	50% ***(b)***	36% ***(d)***
14.	PO	20% ***(c)***	30% ***(c)***	48% ***(c)***	26% ***(c)***
15.	PQ	51% ***(b)***	66% ***(b)***	67% ***(b)***	59% ***(b)***
16.	PR	48% ***(a)***	50% ***(a)***	52% ***(a)***	54% ***(a)***
17.	PT	47% ***(a)***	63% ***(a)***	46% ***(b)***	55% ***(b)***
18.	PU	39% ***(a)***	48% ***(a)***	38% ***(b)***	33% ***(a)***
19.	PV	40% ***(b)***	50% ***(b)***	53% ***(a)***	53% ***(b)***
20.	PX	44% ***(c)***	-	43% ***(c)***	48% ***(c)***

Pune. Genomic DNA was isolated by the standard phenol/chloroform extraction method, followed by PCR amplification of the ITS regions using universal primers ITS1 [5′-TCC GTA GGT GAA CCT GCG G -3′] and ITS4 [5′-TCC TCC GCT TAT TGA TAT GC-3′]. The amplified ITS PCR product was purified by PEG-NaCl precipitation and directly sequenced on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) as per the manufacturer's instructions. Essentially, sequencing was carried out from both ends so that each position was read at least twice. Assembly was carried out using Lasergene package followed by NCBI BLAST against sequences from type material for tentative identification (Boratyn et al. 2013). All the retrieved and tested sequences were aligned using the ClustalW program and subjected to phylogenetic analysis. The phylogenetic tree was constructed using the neighbor-joining (NJ) method in MEGA-X version 10.1.7 with 1000 bootstrap replications and the evolutionary distances were calculated by using the Jukes–Cantor model.

Results and discussion

The present work was carried out on isolated rhizospheric fungal strains and their antagonistic effects on phytopathogens. The rhizosphere fungal isolates were *Penicillium mallochii, Penicillium* sp. FKI-4429, *Cladosporium pseudocladosporioides, Metarhizium anisopliae, Fusarium tricinctum, Cladosporium* sp. MBC003, *Penicillium citrinum, Aspergillus chevalieri, Mucor irregularis, Aspergillus versicolor, Epicoccum nigrum, Aspergillus udagawae*, and *Schizophyllum commune.* They were tested against the phytopathogens *Macrophomina phaseolina*, *Nigrospora oryzae, Boeremia exigua*, and *Phomopsis* sp. PhSFX-1. These were identified according to their cultural, morphological, microscopical, and genetic characteristics. This study has provided useful information about the pathogenic fungi associated with *Macrotyloma uniflorum* plant parts which may affect the plant health, agricultural production, and also economic loss. Also, the antagonistic activity of potent fungi isolated from the rhizosphere region of *Macrotyloma uniflorum* plant against the phytopathogens.

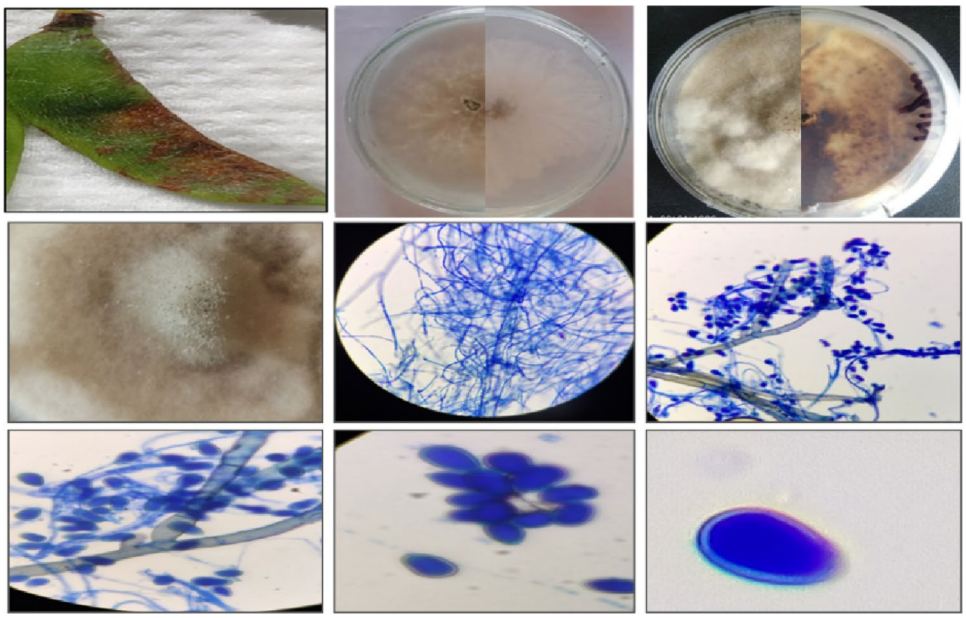


Fig. 3 *Nigrospora oryzae* (left to right) infected seed pod; 3 days old culture, front, back; 7 days old culture, front, back; close view of hyphae; microscopic view of hyphae at 10×, spores at 10×; close view of spores

Isolation and pathogenicity assay of myco-phytopathogens

After 15 days of incubation, out of 10 strains, 4 fungal strains were observed repeatedly which were named as PP 101, PP 102, PP 103, and PP 104 and further preceded.

Symptoms of rot diseases in the field were black, brown, and grey-colored rings. The infected leaves were initially light spotted. Later, they were brownish and slightly wrinkled to wither and eventually die. The dark patches were present on the infected stems and they dried eventually. The seed pods were also infected and seeds were less in numbers in the infected pods as compared to the healthy ones.

On Potato Dextrose Agar media, the morphology of all the isolates was different. Purely isolated cultures of IsolatePP101 have macroscopic features of grey colonies, filamentous resembling cotton, and spreading growths and forming sclerotia. The results of microscopic observations showed that isolatePP101 had elliptical-shaped spores, and branched and aseptate hyphae. IsolatePP102 has black colonies, filamentous like cotton, elliptical spores, and branched and septate hyphae. IsolatePP103 has black colonies with powdery and thread-like structures forming sclerotia and septate hyphae, and isolatePP104 has white-colored colonies with cottony wavy texture, spherical-shaped spores, filamentous, and septate hyphae.

All the plants that were injected with isolates for pathogenic test were infected with rot diseases (Fig. 1). The

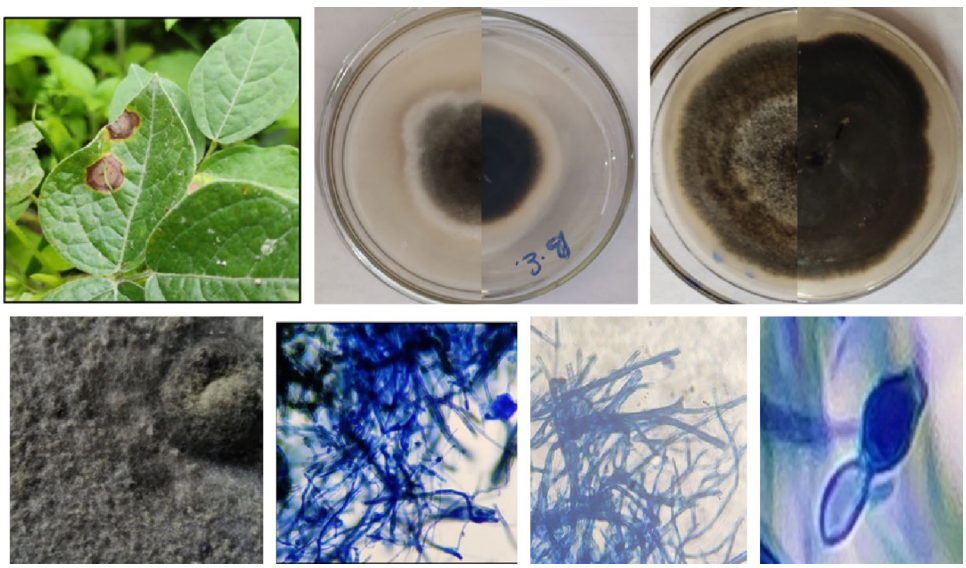


Fig. 4 *Boeremia exigua* (from left to right) infected plant leaf; 3 days old culture, front, back; 7 days old culture, front, back; close view of hyphae; microscopic view of hyphae at 10×; spores at 10×; close view of spores

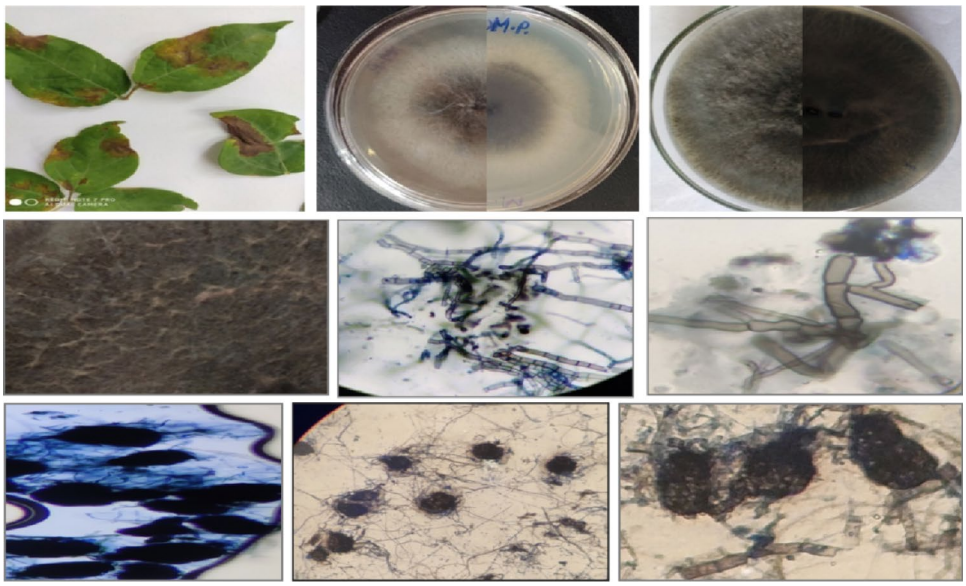


Fig. 5 *Macrophamina phaseolina* (from left to right) infected plant leaf; 3 days old culture, front, back; 7 days old culture, front, back; close view of hyphae; microscopic view of hyphae at 10×; hyphae at 40×; sclerotia; picnidiai at 10×, 40×

pathogens recovered from the experimented plants were morphologically similar to the isolates. This experiment confirmed the pathogenicity of the isolated strains. The symptoms of the rot disease with black and brown spots were observed on leaves, pods, and stem part of *Macrotyloma uniflorum* plant.

Isolation and antagonistic assay of rhizosphere fungal strains against myco-phytopathogens

A total of thirty rhizosphere fungal isolates were recovered among which fifteen strains showed potent antagonistic activity against the myco-phytopathogens (Fig. 2). The results of the antagonist test obtained fifteen isolates that were able to inhibit the isolated pathogens PP101, PP102, PP103, and PP104 (Table 1).

Calculation

Antagonistic Index %:

$$\frac{RM - rm \times 100}{RM}$$

RM: radius of the pathogen in the control plate.
rm: radius of the pathogen in the dual culture plate.

The potent antagonistic fungal strains were PA, PB, PC, PD, PF, PG, PH, PL, PM, PN, PR, PT, PV, and PX which later on were identified as *Penicillium mallochii, Penicillium* sp. FKI-4429, *Cladosporium pseudocladosporioides, Metarhizium anisopliae, Fusarium tricinctum, Cladosporium* sp. MBC003, *Penicillium citrinum, Aspergillus chevalieri, Mucor irregularis, Aspergillus*

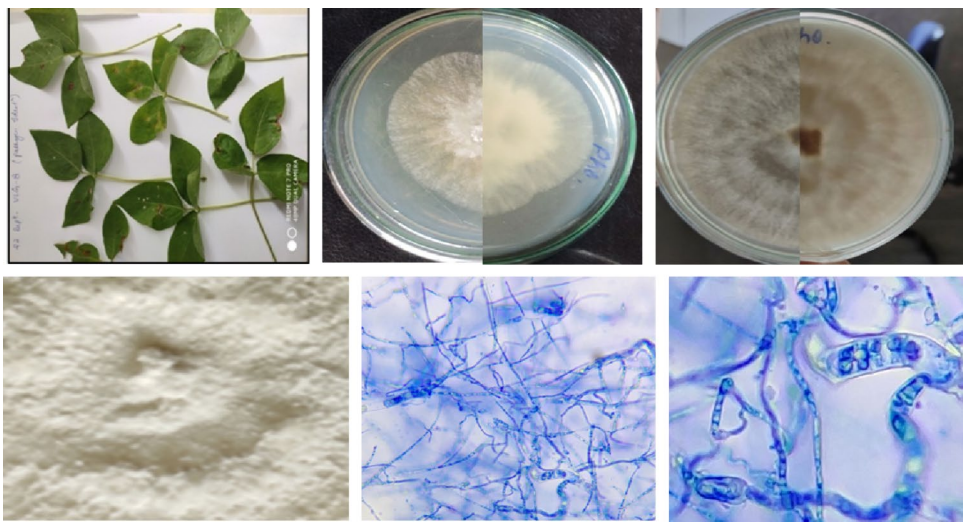


Fig. 6 *Phomopsis* sp. PhSFX-1 (from left to right) infected plant leaf; 3 days old culture, front, back; 7 days old culture, front, back; close view of hyphae; microscopic view of hyphae at 10×

Table 2 Morphological characteristics and molecular identification of pathogenic fungi

S. no	Identity	Colony color	Group	Texture	Spore shape	Septate	Identified as	% Similarity	Accessions	Accessions no. obtained from NCBI
1	PP101	Black/grey	Didymellaceae	Cottony	Elliptical	Aseptate	*Nigrospora oryzae*	100%	KX219801.1	OK244648.1
2	PP102	Black	*Botryosphaeriaceae*	Cottony	Elliptical	Septate	*Boeremia exigua*	100%	MH550515.1	ON791481
3	PP103	White/ Grey	Apiosporaceae	Powder	Round	Septate	*Macrophamina phaseolina*	100%	HQ392782.1	OK244650.1
4	PP104	White	Trichosphaeriaceae	Wavy	Spherical	Septate	*Phomopsis* sp. PhSFX-1	100%	MH371253.1	OK244651.1

versicolor, Epicoccum nigrum, Aspergillus udagawae, and *Schizophyllum commune*. The rest of the fungus did not show antagonistic activity against the phytopathogens. Among these fungal isolates for *Macrophomina phaseolina* pathogen, isolatePM (55%) showed maximum fungal mycelial growth inhibition while isolatePO (20%) showed the least inhibition similarly for *Nigrospora oryzae* IsolatePQ (66%) showed maximum growth inhibition while IsolatePC (23%) the least inhibition; for *Boeremia exigua* IsolatePF (60%) showed maximum growth inhibition while IsolatePD (20%) showed the least inhibition, and for *Phomopsis* sp. PhSFX-1 IsolatePH (60%) showed maximum growth inhibition while IsolatePG (23%) showed the least inhibition as shown in Table 1. Isolates that showed fungal mycelial growth inhibition of more than 40% against isolated phytopathogens were further selected for molecular identification. The natural capacity to suppress pathogens has been studied in many disease-suppressive soils against the oomycetes and fungi *Pythium ultimum*, *Pythium irregulare*, *Pythium aphanidermatum*, *Phytophthora nicotianae, Phytophthora capsici*, *Phytophthora cinnamomi*, *Rhizoctonia solani*, and *Fusarium oxysporum*. The understanding of disease suppressive mechanisms is a crucial step to enhance the suppressive effect by manipulation of the soil microbiota. More specifically, the suppressive properties can be explained through combined antimicrobial actions exerted by molecules and microbes or mechanisms of antagonism among microbes and pathogens. The biological factors based on disease suppression generally include a combination of different actions. The mechanisms underlying the suppressive effect are primarily associated with the biological activity of soil microbiota which interacts with the soil organic matter (SOM) as well as the host plant. The most important factors are represented by the increased microbial activity (Melero et al. 2006) and fungistasis (Bonanomi et al. 2017), enhanced soil structure (Bronick and Lal 2005), release of mineral nutrients during SOM decomposition (Berry et al. 2002), activation of competition for space and nutrients (Noble and Coventry 2005), elicitation of microbiostasis and hyperparasitism, release of diffusible antibiotic-like compounds (Weller et al. 2002), and activation of systemic disease-resistance in the host plant (Bulluck Iii et al. 2002). A direct inhibition on conidial germination and mycelium growth of plant pathogens induced by *Bacillus*, *Pseudomonas*, *Streptomyces*, *Trichoderma*, and *Penicillium* has been documented against phytopathogenic fungi using compost water extract (El-Masry et al. 2002; McQuilken et al. 1994). Suppression of *Fusarium melonis* in wilt-suppressive soils and composted green wastes-amended soils has been associated with populations of *Aspergillus*,

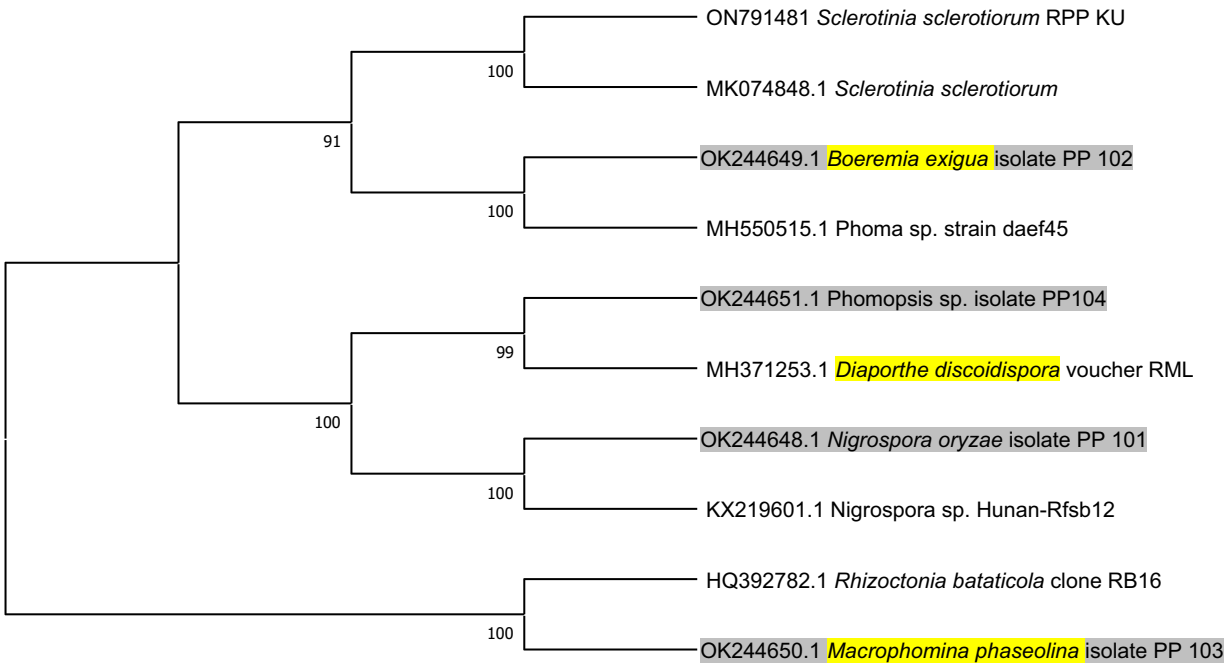


Fig. 7 Phylogenetic tree based on neighbor-joining analysis of the rDNA ITS sequences of the pathogenic fungal isolates obtained from various tissues of the *M. uniflorum* plant. The pathogenic fungal isolates along with their obtained accession numbers are highlighted. For the closely related species, the taxonomic names are written with their respective accession number. Significant bootstrap values (> 50%) are indicated at the branching points

Streptomyces, and fluorescent *Pseudomonas* (Cha et al. 2016; Suàrez-Estrella et al. 2007). Sewage sludge compost suppresses *F. oxysporum* sp. *melonis* wilt on tomato if combined with selected *Trichoderma asperellum* isolates (Cotxarrera et al. 2002). Other authors instead concluded that species of *Penicillium* can act as top BCAs against *Fusarium oxysporum* sp. *lycopersici* of tomato (Hussain et al. 2016).

Based on the analysis of the variety of testing of potential antagonist isolates against the fungus *Macrophomina phaseolina, Nigrospora oryzae, Phomopsis* sp. PhSFX-1, and *Boeremia exigua* on 7 days after inoculation showed that the inoculation of the potential antagonist isolates had a significant effect on the percentage of incidence of phytopathogens fungal disease in vitro. The following is presented about the average percentage of incidence of pathogenic fungal disease in vitro in Table 1.

Microscopic and molecular characterization of myco-phytopathogens

The fungi isolated were examined morphologically, and their spores and thallus structure were analyzed under the compound microscope (Figs. 3, 4, 5, 6). The detailed characteristic features of myco-phytopathogens are shown in Table 2.

The selected phytopathogens were sent for molecular identification based on ITS sequencing. Pathogenic fungi PP101, PP102, PP103, and PP104 were closely related to *Nigrospora oryzae, Boeremia exigua, Macrophamina phaseolina,* and *Phomopsis* sp. PhSFX-1 and showed 100% identity similarity with accessions KX219801.1, MH550515.1, HQ392782.1, and MH371253.1. These sequences are submitted to the Genbank nucleotide database with accession numbers obtained as OK244648.1, ON791481, OK244650.1, and OK244651.1, respectively, as shown in Table 2.

The evolutionary relationship of all the sequences was determined by constructing a phylogenetic tree (Fig. 7).

These fungi are reported as highly pathogenic strains causing huge harm to the crops. There are many reports on the pathogenicity of *Nigrospora* sp. (Jia et al. 2024; Raza et al. 2010; Wright et al. 2008; Zhao et al. 2014; Dutta et al. 2015), *Boeremia exigua* (Kadir and Umaerus 1987; Gorny et al. 2015; Michel et al. 2018; Grinbergs and France 2014; Michel et al. 2018; Gao et al. 2019; Banerjee and Panja 2020), *Macrophomina phaseolina*

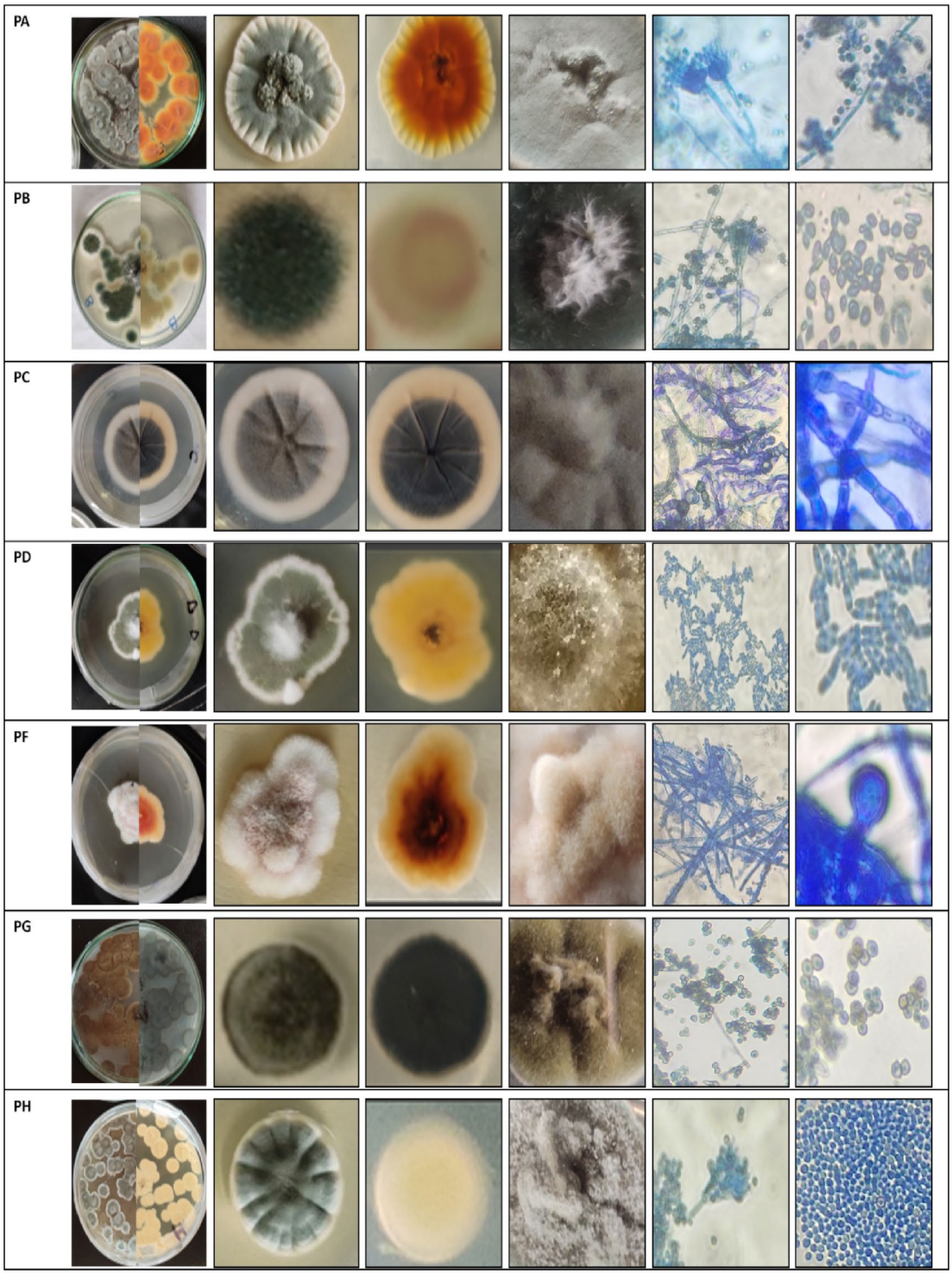


Fig. 8 Rhizospheric fungi isolated from *Macrotyloma uniflorum* plant

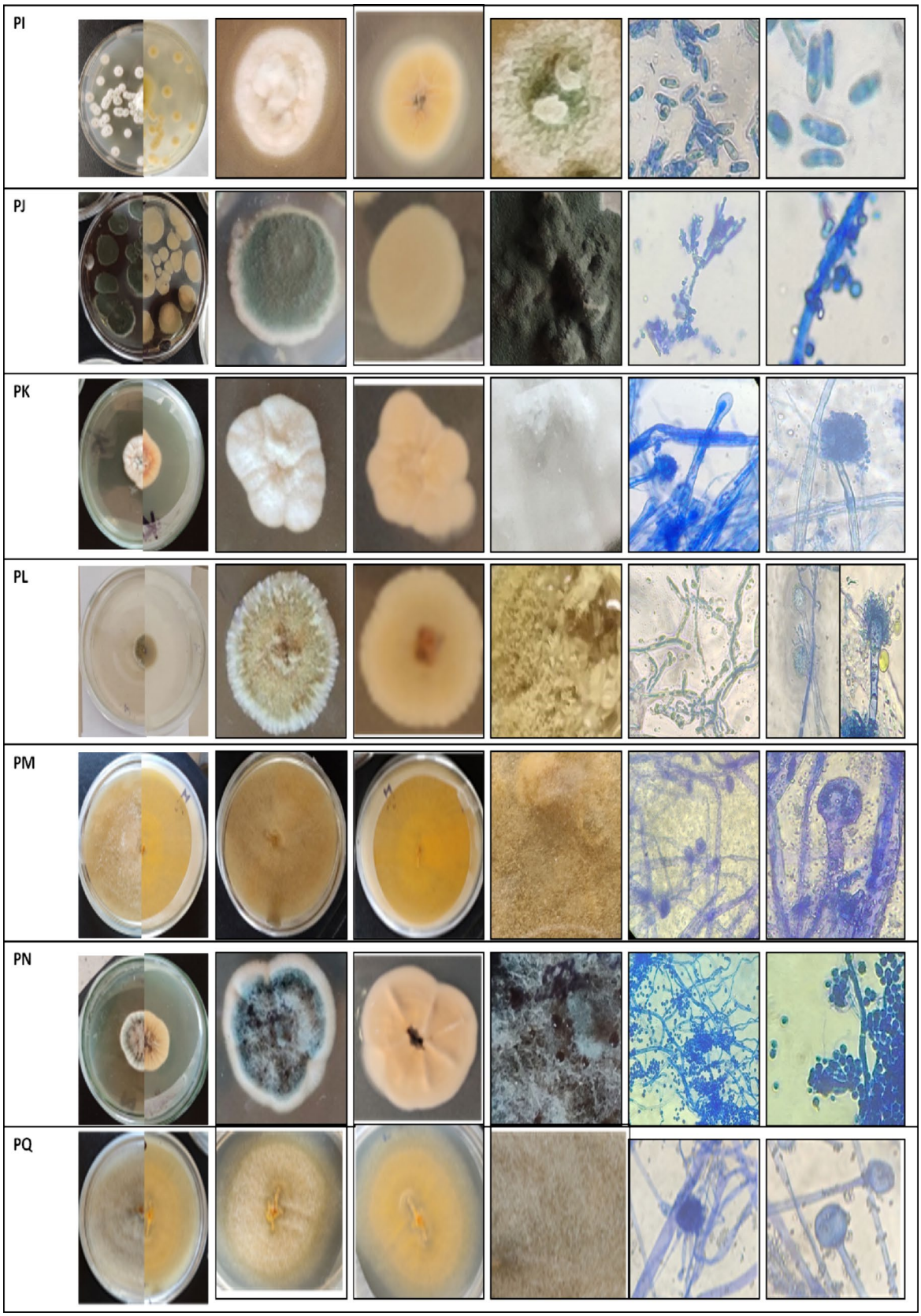


Fig. 8 (continued)

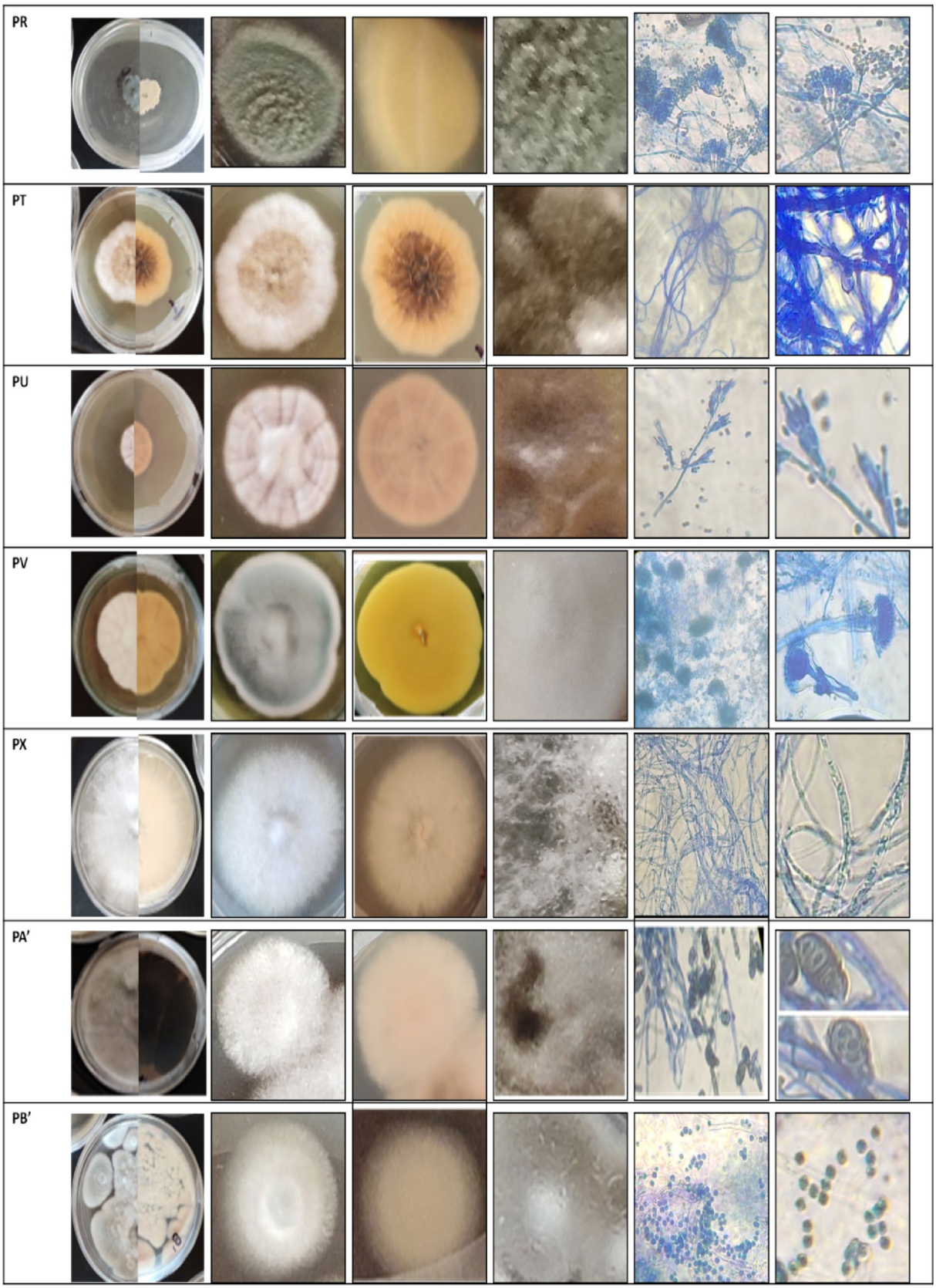


Fig. 8 (continued)

Table 3 Molecular characterization of rhizospheric fungal isolates

Isolates	Identified as	Accessions	% Similarity	Accessions obtained from NCBI
PA	*Penicillium mallochii*	MN944416.1	100%	ON791482
PB	*Penicillium* sp. FKI-4429	AB548364.1	100%	ON791483
PC	*Cladosporium pseudocladosporioides*	MT582794.1	100%	ON791484
PD	*Metarhizium anisopliae*	MN710409.1	100%	ON791485
PF	*Fusarium tricinctum*	MN594466.1	100%	ON791486
PG	*Cladosporium* sp. MBC003	JQ885448.1	99.81%	ON791487
PH	*Penicillium citrinum*	MG748682.1	100%	ON791488
PL	*Aspergillus chevalieri*	MT316337.1	100%	ON791489
PM	*Mucor irregularis*	MZ423089.1	100%	ON791490
PN	*Aspergillus versicolor*	MN547369.1	100%	ON791491
PR	*Penicillium citrinum*	MT558921.1	100%	ON791492
PT	*Epicoccum nigrum*	MT166336.1	100%	ON791493
PV	*Aspergillus udagawae*	MN882827.1	100%	ON791494
PX	*Schizophyllum commune*	MK647986.1	100%	ON791495

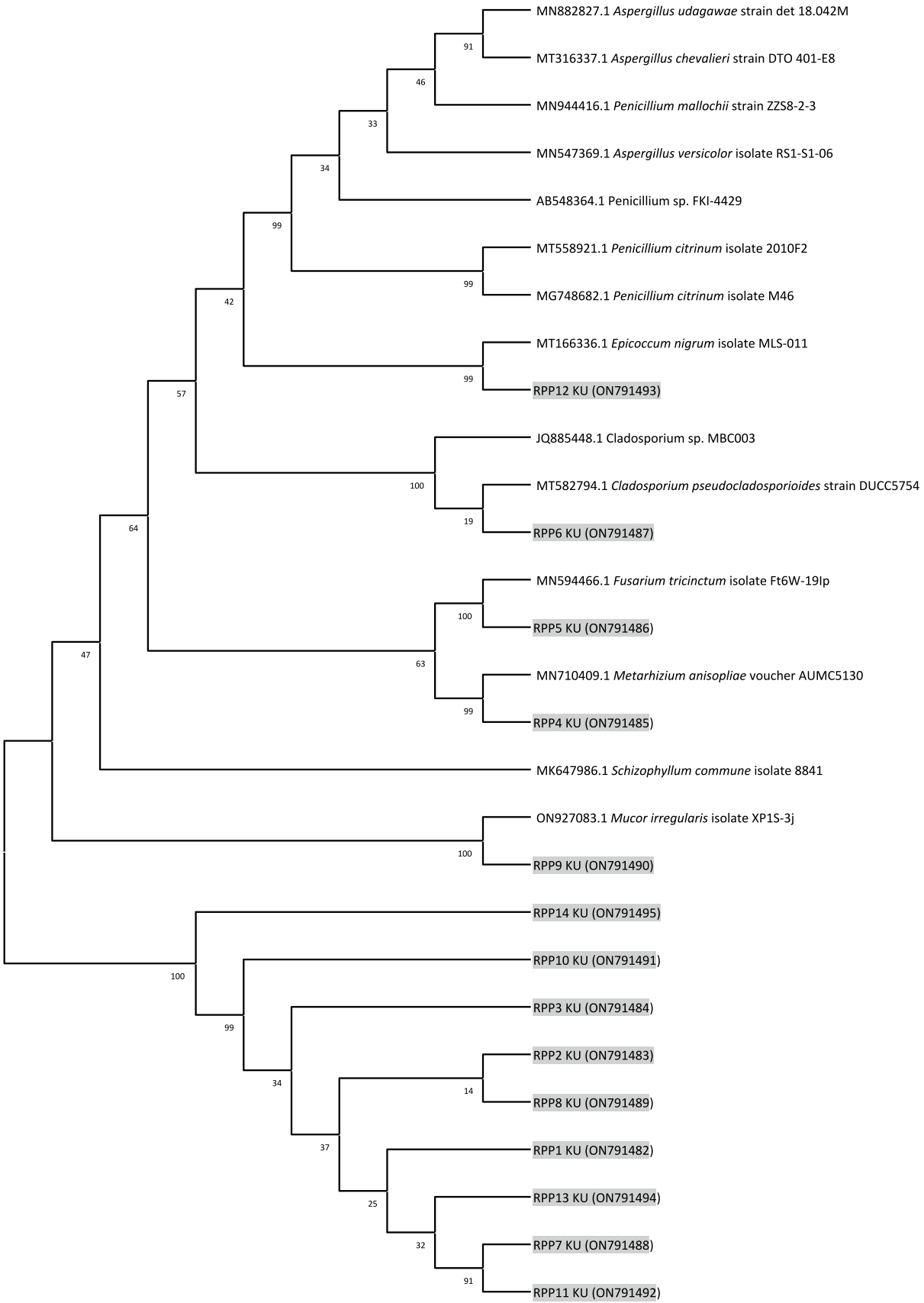
(Basandrai et al. 2021; de Sousa Linhares et al. 2020; Marquez et al. 2021; Teja et al. 2020; Lodha and Mawar 2020), and *Phomopsis* sp. PhSFX-1 (Chaisiri et al. 2020; Anwar et al. 2017; Asad et al. 2015; Correia et al. 2017; Brumer et al. 2018) on the stem, fruit, leaf, and root parts of wheat, potato, lima bean, sugarcane, rice, legumes, sesame, tea, etc. These are very aggressive types of fungal phytopathogens infecting plant health, yield, and growth promotion. The genus of *Nigrospora* is a widely distributed fungus, which can exist as an endophyte and plays a role as a pathogen to affect plant health (Wang et al. 2017; Ebada et al. 2016). The pathogenic fungus, *Boeremia exigua*, infects many other plants as their hosts. For example, 11 varieties of *B. exigua* reportedly infect 45 plant species belonging to 31 genera and 19 families (Berner et al. 2015). *Macrophomina phaseolina* is a soil-borne fungal pathogen that incites charcoal rot in more than 500 plant species (Marquez et al. (2021). *Diaporthe*/*Phomopsis* species are widely distributed around the world; they are pathogens of many important crops and can grow as parasites on humans and animals also (Van Warmelo et al. 1970; Udayanga et al. 2011; Gomes et al. 2013). Pathogenic *Diaporthe* species can grow in plant tissue without causing clearly visible symptoms for a long time. But later, they do kill the host tissue so they should be categorized as hemibiotrophs (Udayanga et al. 2011).

Microscopic and morphological characterization of rhizospheric fungi

The rhizospheric isolates are given in Fig. 8.

Molecular characterization of selected potent antagonist rhizospheric fungi

Among all the tested fungal strains, 15 strains showed some sort of antagonism against the phytopathogens in dual culture assay on PDA media. The fungal isolates that showed partial antagonistic activity against the phytopathogens were sent for molecular identification based on ITS sequencing. The sequences are submitted to the Genbank Nucleotide database with accession numbers given in Table 3. Phylogenetic trees constructed from 16S rRNA sequences (Fig. 9). The phylogenetic tree indicated that isolatePA, PB, PC, PD, PF, PG, PH, PL, PM, PN, PR, PT, PV, and PX were closely related to *Penicillium mallochii, Penicillium* sp. FKI-4429, *Cladosporium pseudocladosporioides, Metarhizium anisopliae, Fusarium tricinctum, Cladosporium* sp. MBC003, *Penicillium citrinum, Aspergillus chevalieri, Mucor irregularis, Aspergillus versicolor, Penicillium citrinum, Epicoccum nigrum, Aspergillus udagawae*, and *Schizophyllum commune* and showed 99–100% identity with accessions MN944416.1, AB548364.1, MT582794.1, MN710409.1, MN594466.1,


MN882827.1 Aspergillus udagawae strain det 18.042M
MT316337.1 Aspergillus chevalieri strain DTO 401-E8
MN944416.1 Penicillium mallochii strain ZZS8-2-3
MN547369.1 Aspergillus versicolor isolate RS1-S1-06
AB548364.1 Penicillium sp. FKI-4429
MT558921.1 Penicillium citrinum isolate 2010F2
MG748682.1 Penicillium citrinum isolate M46
MT166336.1 Epicoccum nigrum isolate MLS-011
RPP12 KU (ON791493)
JQ885448.1 Cladosporium sp. MBC003
MT582794.1 Cladosporium pseudocladosporioides strain DUCC5754
RPP6 KU (ON791487)
MN594466.1 Fusarium tricinctum isolate Ft6W-19Ip
RPP5 KU (ON791486)
MN710409.1 Metarhizium anisopliae voucher AUMC5130
RPP4 KU (ON791485)
MK647986.1 Schizophyllum commune isolate 8841
ON927083.1 Mucor irregularis isolate XP1S-3j
RPP9 KU (ON791490)
RPP14 KU (ON791495)
RPP10 KU (ON791491)
RPP3 KU (ON791484)
RPP2 KU (ON791483)
RPP8 KU (ON791489)
RPP1 KU (ON791482)
RPP13 KU (ON791494)
RPP7 KU (ON791488)
RPP11 KU (ON791492)
91
46
33
34
99
99
42
99
57
100
19
64
100
47
63
99
100
99
34
14
37
25
32
91

◂**Fig. 9** Phylogenetic tree based on neighbor-joining analysis of the rDNA ITS sequences of the rhizospheric fungal isolates obtained from rhizosphere soil of *M. uniflorum* plant. The rhizospheric fungal codes along with their accession numbers obtained are highlighted. For the closely related species, the taxonomic names are written with their respective accession number. Significant bootstrap values (> 50%) are indicated at the branching points

JQ885448.1, MG748682.1, MT316337.1, MZ423089.1, MN547369.1, MT558921.1, MT166336.1, MN882827.1, and MK647986.1, respectively (Table 3).

Conclusion

The present investigation concludes that out of thirty fungal strains, fifteen strains efficiently suppressed the mycelial growth of pathogenic *Macrophomina phaseolina, Nigrospora oryzae, Boeremia* exigua, and *Phomopsis* sp. PhSFX-1 fungi in direct interactions-assays in vitro. The ITS sequence analysis of rhizospheric fungal strains showed 98 to 100% identity with close relatives belonging to *Penicillium mallochii, Penicillium* sp. FKI-4429, *Cladosporium pseudocladosporioides, Metarhizium anisopliae, Fusarium tricinctum, Cladosporium* sp. MBC003, *Penicillium citrinum, Aspergillus chevalieri, Mucor irregularis, Aspergillus versicolor, Epicoccum nigrum, Aspergillus udagawae*, and *Schizophyllum commune.* These fungi have antagonistic potential against pathogenic fungi. These fungal isolates should further be analyzed for metabolomics study to study their active constituents. These results confirmed the significant role of native rhizospheric fungi for the control of soil-borne fungal pathogens and the potential use of identified isolates in biofertilizers and bio-fungicides development.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by P.P. and A.N. The first draft of the manuscript was written by P.P., J.R. and R.K. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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