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Evaluation of *Trichoderma harzianum* as a potent biocontrol agent in suppression of leaf blight disease of *Petrea volubilis*, caused by *Meyerozyma guilliermondii*

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Mature leaves of *Petrea volubilis,* evergreen flowering vine commonly known as Nilmonilata has been found to be infected with foliar fungal pathogen showing leaf blight symptoms. After completion of Kochs'postulate the fungal pathogen has been identified as *Meyerozyma guilliermondii* based on molecular characterization and submitted to NCBI Genbank (PQ467080). In phylogenetic analysis this strain (PQ467080) was found to be closely related with extype of *M. guilliermondii* isolate mYJdd12 (KR632523). Besides, at the onset Hemolytic activity assay was perforemed in order to verify whether the *M. guilliermondii* isolate PV- 3 (PQ467080) was human pathogenic or not. This isolate PV-3 was found to be non human pathogenic . *Trichoderma harzianum* [NAIMCC-F-01961; NCBI GQ995194] a potential biocontrol agent showed 83.5 per cent inhibition *in vitro* against tested fungal pathogen (*M. guilliermondii*). The zone of interaction *in vitro* between mycelia of *M. guilliermondii* and *T. harzianum* was studied by scanning electron microscopy.Wheat bran as well as Farm yard manure based formulation of *T. harzianum* was amended in the rhizosphere as well as foliar application of spore suspension was carried out prior to artificial inoculation of*M. guilliermondii* and evaluated for development of leaf blight disease. *T. harzianum* was found to be effective in reducing leaf blight symptom and control disease more efficiently. In addition,plant growth status was significantly improved. Flowering was also induced following application of FYM based formulation of biocontrol agent.

Keywords : *Petrea volubilis*, Leaf blight disease, *Meyerozyma guilliermondii*, *Trichoderma harzianum*, disease suppression

INTRODUCTION

The flowering vine *Petrea volubilis*, also called Nilmonilata, member of Verbenaceae is well-known for its eye-catching clusters of violet flowers. It has puberulent stems and can grow as a vine or semi-climbing shrub. The thick, textured leaves have an acute apex, a wedge-shaped base, a full border, and occasionally a sinuous, glabrous, or pubescent appearance. Bracts with racemose inflorescence give rise to the flowers (Fig.1).

On mature leaves of *P. volubilis*, appearance of fungal blight symptoms were noticed during the month of July and August in the gradening areas of Upohar Condoville, New Garia, Kolkata (GIS location: 22^{0}28'39"N; 88^{0}24'01"E). The fungal pathogen has been identified as *Meyerozyma guilliermondii* which is a new record from West Bengal, India.However, members of the *Meyerozyma guilliermondii* species complex has been earlier reported as human pathogenic (Corte *et.al.*, 2015; da Matta *et.al.,*2017)

Trichoderma species has been gaining popularity as one of the most important biocontrol agents (Chakraborty *et.al*. 2020). Application of isolated and purified *Trichoderma harzianum* T22 strain in biological control of plant pests and its commercial development of biological control technology was documented by Harman (2000). It is mostly utilized to manage soil-borne illnesses in a variety of plants as well as certain diseases of the leaves and spikes. Plant resistance, nutrient consumption efficiency, growth, and disease prevention are all enhanced by *Trichoderma* (Abbas *et.al.* 2022; Al-Surhanee, 2022; Tyœkiewicz *et al.* 2022, Di Marco *et al.* 2022).

Trichoderma has been used in biological control research, including *T. harzianum*, *T. viride*, *T.*

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asperellum etc. and their mode of action such as competition, antibiosis, mycoparasitism, hyphal interactions and enzyme secretion have been described . Chakraborty *et al.* (2010) reported the isolation of *T. harzianum* and *T. viride* from the rhizospheric soils of plantation crops, agricultural fields of North Bengal and studied in detail about the molecular characterization of those isolates. Different species of *Trichoderma* have reported as potent antagonists against fungal pathogens such as -*Sclerotium rolfsii*, *Rhizoctonia solani, Pythium ultimum, Fusarium oxysporum, Sclerotinia sclerotiorum* etc ((Chakraborty *et al.* 2011, Zhang *et al.* 2022). Rabnawaz *et al.* (2023) also reported the role of *T. harzianum* in plant growth improvement and in suppression of leaf blight disease. Biocontrol potential of *T. harzianum* by inducing biochemical changes in *Persea bombycina* plant, has been reported (Rabba *et al.* 2014).

Keeping these in view, the present study has been undertaken in order to evaluate the application of *Trichoderma harzianum* as biocontrol agent in suppression of a leaf blight disease of *Petrea volubilis* caused by *Meyerozyma guilliermondii*, a new record from West Bengal, India. However, at the onset test was performed to verify whether our *M. guilliermondii* isolate coded as PV 3 was human pathogenic or not.

MATERIAL AND METHODS

Isolation of pathogen from infected leaf of Petrea volubilis showing blight disease

The diseased leaves having blight symptoms (1 to 1.5 cm) were cut into small pieces and transferred to potato dextrose agar (PDA) medium after surface sterilization of the leaf sample with 0.1% $HgCl_2$ for 2 min, followed by ethanol for 2 min. A fungus was isolated from the infected leaf onto potato dextrose agar amended with the antibiotic Monocef-O 100 (Each 5 ml of the suspension contained cefpodoxime proxetil IP equivalent to 100 mg of cefpodoxime).

Morphological and molecular identification of fungal pathogen

The pathogen was characterized morphologically by using light and scanning electron microscope. Cream-colored mature mycelia and spherical to sub spherical like cells with branched hyphae of the causal pathogen were observed. For molecular methods, DNA was isolated from the culture. Quality was evaluated on 1.8% agarose gel and a single band of high-molecular weight DNA was observed. Isolated DNA was amplified with 18s rRNA specific primer (ITS1- TCC GTA GGT GAA CCT GC GG and ITS4- TCC TCC GCT TAT TGA TAT GC) using Veriti® 96 well Thermal Cycler. A single discrete PCR amplicon band of ~600 bp was observed. The PCR amplicon was purified and bi-directional DNA sequencing reaction of PCR amplicon was carried out with ITS1 and ITS4 primers using BDT v3.1 Cycle sequencing kit on ABI 3500Dx Genetic Analyzer. Phylogenetic analysis was performed by neighbor joining (NJ) method (Saitou and Nei, 1987) through MEGA 11 software (Tamura *et al.* 2021). 18S rDNA of *Meyerozyma guilliermondii* (PQ467080) was aligned to study the range of homology present in the conserved regions following the ClustalW algorithm (Thompson *et al.* 1994) using the Bioinformatic tool BioEdit. Combinations and percentage of occurrence of different nucleotide in the entire sequence of *Meyerozyma guilliermondii* were calculated using the bioinformatics algorithm from the website:

http://www.ualberta.ca/~stothard/javascript/dna_stats.html.

Hemolytic activity assay

Hemolytic activity assay was done for verifying whether the *M. guilliermondii* isolate PV- 3 was human pathogenic or not. The assay was performed on Malt extract agar supplemented with 5% goat blood. The agar was poured into 20 ml volumes into plastic plates. PV-3 isolate was inoculated and grown aerobically at 30 °C for 48 h. Hemolytic activity was monitored every 12h (Aktas and Yigit 2015). Isolate would be considered hemolytic if, in this test, zones or rings of alpha- and/or beta -hemolysis were observed in the growth areas or adjacent to the colonies after the incubation period. The assay was performed three times for confirmation.

Koch's postulate on detached leaves of Petrea volubilis

Leaf blight symptoms were developed within 7 days on inoculated detached mature leaves of

Fig 1: *Petrea volubilis* flowering in Upohar Complex

Fig 2:(A) Leaf blight disease symptoms of *Petrea volubilis* inUpohar Complex **(B)** GIS location:22^{0}28’39"N; 88^{0}24’01"E of appearance of disease

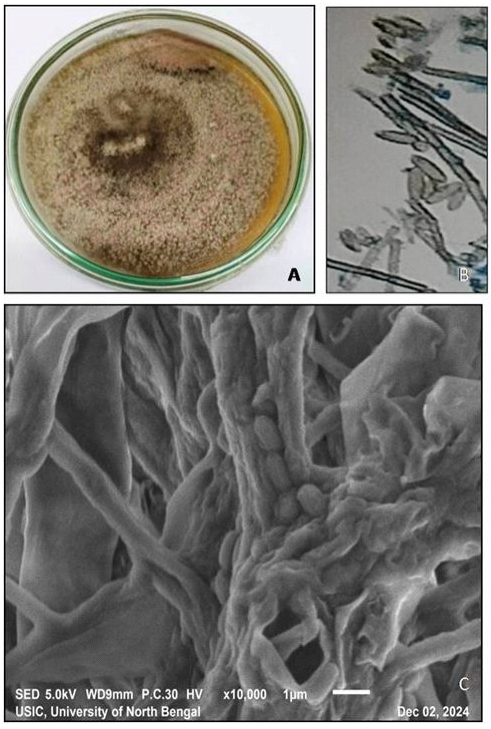

Fig 3 : *Meyerozyma guilliermondii* (causal organism of Leaf blight of *Petrea volubilis*) (A) Mycelial growth with sporulation, (B) Hyphae and conidia under bright field microscope and (C) Hyphae and conidia under scanning electron microscope

P. volubilis after application of spore suspension ($1x10^3$ spores/ml) of the isolated fungal pathogen whereas control leaves remained unaffected. The fungal pathogen was re-isolated from the blight region of the leaves and morphology of the foliar pathogen was reconfirmed through Koch's postulate.

Source of culture of Trichoderma harzianum

Trichoderma harzianum was identified molecularly and submitted to NCBI Genbank [GQ995194]. The culture was identified during the NBAIM project work at the Immuno-Phytopathology Laboratory, NBU and then submitted to the culture deposition of NBAIM under the accession number- NAIMCC-F-01961. During present study, the culture was collected from NBAIM.

In vitro antagonistic test of T. harzianum against the leaf blight pathogen

The *in vitro* antagonistic activity of *T. harzianum* against leaf blight pathogen was examined. An agar disc of the test fungus was positioned 2.5 cm apart from *T. harzianum* on the medium. A negative control was also available, which only deployed fungal agar discs devoid of any *Trichoderma* culture spots. The inoculation plate was incubated for five days at 28 ± 1 °C to measure the colony growth inhibition. The percentage of inhibition was calculated using the formula PI= [(R1-R2) / R1] X 100 (where PI stands for percentage of inhibition, R2 for radial growth of the fungus in the control plate and R1 for radial growth of the fungal colony opposite the *Trichoderma* culture).

Scanning electron microscopy of mycelia of leaf blight pathogen and spore of T. harzianum during in vitro antagonism

Scanning electron microscopy was performed in order to study the transition zone between the mycelia of leaf blight pathogen and spore of *T. harzianum*. The inoculums from the transition zone were put on a black adhesive tape by using a scalpel and then gold coating procedure was performed. Scanning electron microscopy was carried out with a JSMIT-100 model at the USIC centre, NBU.

Preparation of wheat bran based formulations of T. harzianum

2.5g of carboxymethyl cellulose was added to 250 g of wheat bran and pH was adjusted to 7 by adding calcium carbonate. They were sterilized for 30 min in two consecutive days. Concentration ($1x10^3$ spores/ml) of spore suspension of *T. harzianum* was used as inoculums. The wheat bran mix was dried under shade to bring moisture to less than 20 %. The formulation was packed in milky white color polythene bags, sealed and stored at room temperature for future use.

Application of formulations of T. harzianum

To test *in vivo* efficacy of wheat bran based formulation of *T. harzianum* for plant growth promotion of *Petrea volubilis*, fresh formulations were applied in potted soil at the rate of 100g/pot. Plants were grown under natural conditions of light and temperature. For determining the growth promotion of the plant grown in the field following application of bioinoculant, increase in height, number of leaves were noted.

Disease assessment

Plants were inoculated with *M. guilliermondii* and 45 days after inoculation disease development was assessed using 0-5 scores. Disease severity scale for leaf blight disease was followed as described by Cahudhary, 1996. Average of 10 plants/treatment was considered. Disease score ranging from 0-5 scale was noted for leaf blight development as follows:[0=No lesion : Category:Highly Resistant (HR);1=1-10% lesion area : Category: Resistant (R);2=11-30% lesion area : Category: Moderately Resistant (MR);3=31-50% lesion area : Category: Moderately Susceptible (MS);4=51-75% lesion area : Category : Susceptible (S); 5=76-100%lesion area : Category: Highly Susceptible (HS)].

RESULTS

Emergence of leaf blight symptoms on mature leaves of Petrea volubilis

Fungal leaf blight disease was found on the leaves of *P. volubilis* plants in July and August 2024.

Initially, the symptoms appeared as circular necrotic sores on leaves. There was a dark brown border around the lesions. When these lesions merged, larger grey areas were seen (Fig. 2A**)**. For the foliar pathogen that causes leaf blight to flourish, temperatures between 28^0 and 30°C and a recommended relative humidity of 80% were optimal.

Isolation of fungal pathogen from diseased leaves and study of morphology of the pathogen

The causal pathogen was isolated from diseased leaves on PDA medium and cream-colored mature mycelia were observed on the PDA plate (Fig. 3A). The pathogen was characterized morphologically by using light (Fig 3B) and scanning electron microscope. Spherical to sub spherical like cells with branched hyphae were found during observation under scanning electron microscopy (Fig. 3C).

Establishment of leaf blight symptoms through Koch's postulate

Similar leaf blight symptoms were developed and *Meyerozyma guilliermondii* was re-isolated from the blight region of the artificially inoculated leaves. Although the method of inoculation was artificial, leaf blight symptoms were similar to those observed in field grown plants of *P. volubilis* (Fig. 4 A-D).

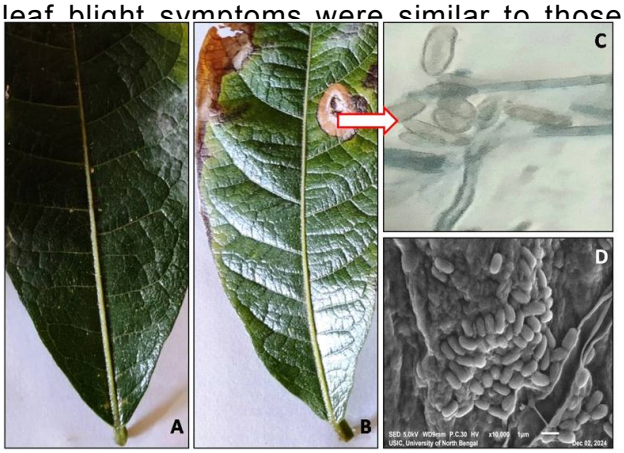

Fig 4 : Completion of Kochs' postulate : (A) Healthy leaf (B) Symptoms developed on Leaf of *Petrea volubilis* after artificially inoculation with *Meyerozyma guilliermondii* (C) Reisolated pathogen- *M. guilliermondii* under bright field microscope and (D) *M. guilliermondii* under scanning electron microscope

Molecular identification of the fungal pathogen

The BLAST query of 18S r DNA sequence of the isolate against GenBank database confirmed its

Fig 5: (A) Agarose gel showing single 600 bp of 18S amplicon of *Meyerozyma guilliermondii* ; (B) 18S rRNA gene sequence of *Meyerozyma guilliermondii* submitted to NCBI Genbank - PQ467080

Table 1: Identified *Meyerozyma guilliermondii* (PV3) and comparison with referred NCBI GenBank

Strain No	GenBank accession no	Identified as	Country of Origin	Identity (%)
LY26	KP053247.1	*Meyerozyma guilliermondii*	India	99
C-650	KX580709.1	*Meyerozyma guilliermondii*	Turkey	99
A1S10Y1	KF268351.1	*Meyerozyma guilliermondii*	India	99
0H7	KF619545.1	*Meyerozyma guilliermondii*	Kenya	99
18H1	KF619550.1	*Meyerozyma guilliermondii*	Kenya	97
WM10.14	JN183444.1	*Meyerozyma guilliermondii*	Australia	98
YN5	KJ502664.1	*Meyerozyma guilliermondii*	China	99
SMB B-G-MA4	HM588762.1	*Meyerozyma guilliermondii*	Republic of Korea	99
AUMC 7771	JQ425356.1	*Meyerozyma guilliermondii*	Egypt	98
VB7	JF730118.1	*Meyerozyma guilliermondii*	India	99
AUS_LFB_3Y_ML_MA	JF921976.1	*Meyerozyma guilliermondii*	India	99
AUS_LFB_2Y_ML_MA	JF921975.1	*Meyerozyma guilliermondii*	India	99
AUS_LFB_1Y_ML_MA	JF794774.1	*Meyerozyma guilliermondii*	India	99
YCH155	KM982965.1	*Meyerozyma guilliermondii*	Brasil	98
SMB B-G-PYD12	HM588764.1	*Meyerozyma guilliermondii*	Republic of Korea	99
KUC5325	KP288249.1	*Meyerozyma guilliermondii*	Korea	99
C-787	KX580710.1	*Meyerozyma guilliermondii*	Turkey	99
MBTU MYW1	JN128648.1	*Meyerozyma guilliermondii*	India	98
mYJdd6i	KR399999.1	*Meyerozyma guilliermondii*	India	97
mYJdd12	KR632523.1	*Meyerozyma guilliermondii*	India	99.91
SW236	KC178873.1	*Meyerozyma guilliermondii*	China	99
CBS 9840	NG_064893.1	*Meyerozyma athensensis*	USA	97
PV 3	PQ467080.1	*Meyerozyma guilliermondii*	India	100
NRRL Y-2075	NG_063363.1	*Meyerozyma guilliermondii*	USA	98
MguEd003	KJ126853.2	*Meyerozyma guilliermondii*	Mexico	99

Table 2: *In vitro* pairing of *Trichoderma harzianum* with *Meyerozyma guilliermondii* for evaluation of antifungal activity

Interacting Microorganisms	Diameter of fungal colony after 7 days growth (cm)[a]	% of Inhibition
Meyerozyma guilliermondii	8.8±0.14	-
M. guilliermondii + *T. harzianum* (NAIMCC-F-01961)	1.5±0.09	83.5±1.82

[a]Values are mean of three replicate experiments.±= Standard Error.

Table 3 : Effect of application of *Trichoderma harzianum* on growth of *Petrea volubilis*

Plant	Treatment	% increase in height after			% increase in number of leaves after		
		15 days	30 days	45 days	15 days	30 days	45 days
Petrea volubilis	Untreated Healthy	20.0±1.2	31.0±2.88	43.0±1.27	11.0±1.15	25.0±0.692	30.0±0.577
	Treated with *Trichoderma harzianum*	41.0±1.154	55.0±0.115	62.0±0.346	33.3±1.64	38.0±1.154	41.5±0.69

Mean of 10 replicate plants / treatment; Differences between control and treatments significant at P=0.05

Table 4 : Evaluation of Leaf blight disease development of *Petrea volubilis* following application of *Trichoderma harzianum*

Plant *Petrea volubilis*	Leaf blight disease index[a]	
	Untreated Healthy inoculated with *M.guilliermondii*	Treated with *Trichoderma harzianum* and inoculated with *M. guilliermondii*
Disease score	5	1
% of leaf area affected	86.2	9.5
Category	Highly Susceptible (HS)	Resistant (R)

[a]Average of 10 plants /treatment – 45 days after inoculation with *M. guilliermondii*
Disease score: 0-5
0=0%lesion area; disease reaction= Highly Resistant (HR), 1=1-10%lesion area; disease reaction= Resistant (R)
2=11-30%lesion area; disease reaction= Moderately Resistant (MR),
3=31-50%lesion area; disease reaction= Moderately Susceptible (MS)
4=51-75%lesion area; disease reaction= Susceptible (S), 5=76-100%lesion area; disease reaction= Highly Susceptible (HS)

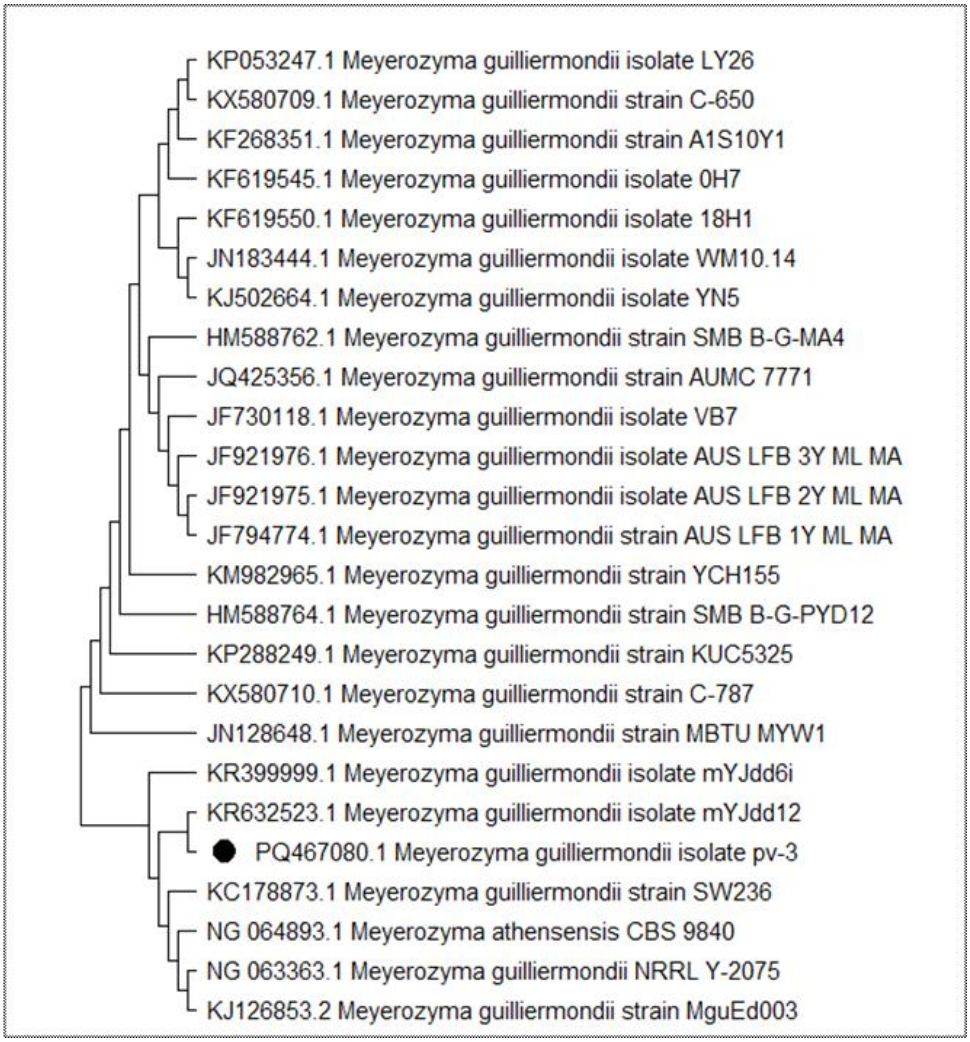

Fig 6: Phylogenetic tree of *Meyerozyma guilliermondii* (PQ467080), closely related to *Meyerozyma guilliermondii* strain mYJdd12 (KR632523)

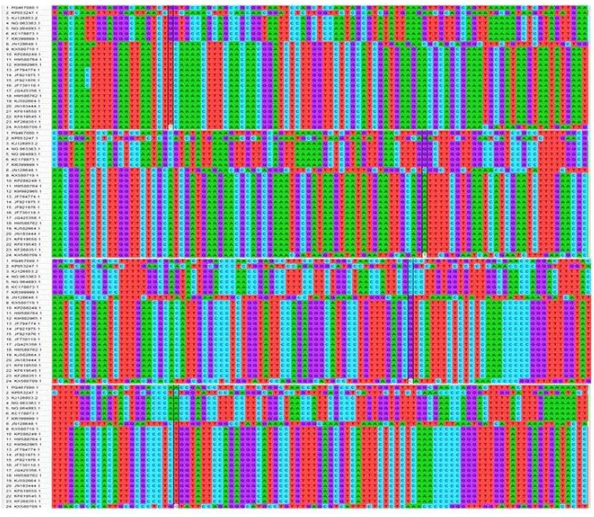

Fig 7 : Multiple sequence alignment of *Meyerozyma guilliermondii* (PQ467080) with other ex types

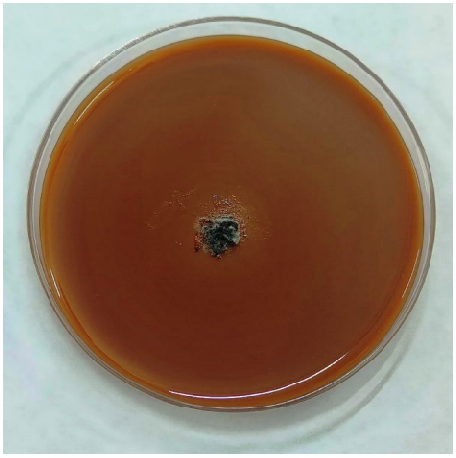

Fig 8 : Haemolytic activity assay of *Meyerozyma guilliermondii* (coded as PV-3) on Malt extract aga supplemented with 5% goat blood. No distinct clear zone surrounding the mycelia block on blood medium. the incidence of no haemolysis indicating the fungus is non human pathogenic strain.

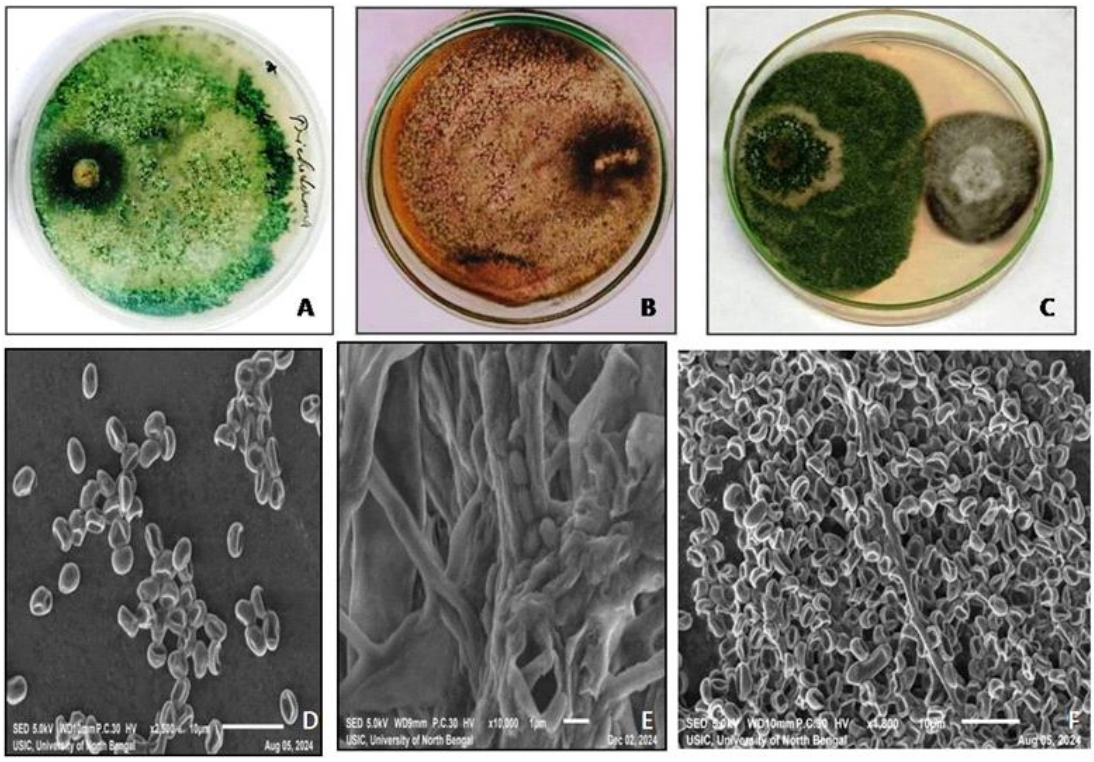

Fig 9 : *In vitro* study on PDA medium **:** (A) *Trichoderma harzianum* **,** (B)*Meyerozyma guilliermondii* and (C) *In vitro* interaction between *T. harzianum*and *M. guilliermondii.* Scanning electron microscopic study of (A) *T. harzianum,* (B) *M. guilliermondii* and (F) Interacting zone between *T. harzianum* and *M. guilliermondii*

Fig. 10 : (A) *Trichoderma harzianum* **(**NAIMCC-F-01961); (B) FYM based formulation of *T. harzianum* ; (C&D) Wheat bran based formulations of*T. harzianum*

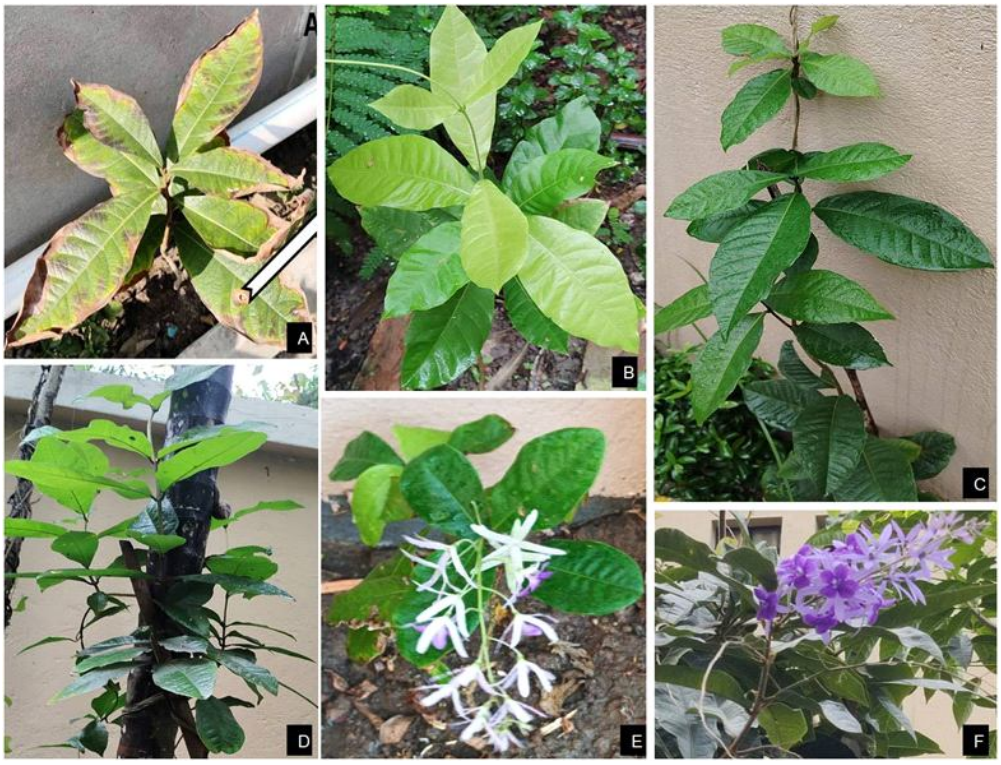

Fig. 11. Growth of *Petrea volubilis* at different stages(A) Untreated plant of artificially inoculated with *Meyerozyma guilliermondii* (B-F) Plant Growth of different stages following application of *Trichoderma harzianum* and challenge inoculation with *Meyerozyma guilliermondii* (E&F) Plant Growth, induced flowering and resistance toward *M. guilliermondii*; following treatment of biocontrol agent (*T. harzianum*, NAIMCC-F-01961).

identity. The fungal pathogen was identified as *Meyerozyma guilliermondii* (PQ467080) based on molecular identification and submitted to NCBI Genbank (Fig. 5). The sequenced PCR product was aligned with extype isolate sequences from NCBI GenBank for identification as well as for studying phylogenetic relationship with other ex-type sequences.

Phylogenetic analyses

The phylogenetic analyses conducted using the NJ method among the isolates of *Meyerozyma guilliermondii* with other ex-type strains obtained from NCBI GeneBank database by MEGA 12 software. In phylogenetic analysis, *M. guilliermondii* (PQ467080) strain was found to be closely related with extype of *Meyerozyma guilliermondii* isolate mYJdd12 (KR632523) (Fig. 6).The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option).

Multiple sequence alignment

Multiple sequence alignment revealed that there were regions in the sequences which were not similar and, hence, gaps were introduced in these regions. Presence of regions with similar sequences indicated relationships among the isolates of *M. guilliermondii*. The conserved regions of the gene were demonstrated in different colour (Fig. 7; Table 1).

Hemolytic activity assay

No distinct halo zone was observed around the mycelial block of *M. guilliermondii* (isolate PV3) on blood agar that indicated the incidence of no hemolysis by the fungal isolate. As no hemolysis was found (Fig.8), so, our *M. guilliermondii* isolate PV- 3 (PQ467080) was found to be non human pathogenic strain.

In vitro antagonism of Trichoderma harzianum against Meyerozyma guilliermondii

The antagonistic effect of *Trichoderma harzianum* against *Meyerozyma guilliermondii* was tested by dual culture methods in solid medium. In solid medium, inhibition of the growth of fungal pathogen and the zone of inhibition were recorded (Fig. 9 A-C). The result revealed that *T. harzianum* inhibited the growth of *M. guilliermondii* significantly. The tested fungus was inhibited to some degree; the percentage inhibition was 83.5% by *T. harzianum* (Table 2).

Interactions of the antagonist- *T. harzianum* and fungal pathogen - *M. guilliermondii* were also studied with the help of scanning electron microscopy.The SEM micrographs revealed that the *Trichoderma* spore parasitizes the pathogen mycelium and inhibits its growth.The pathogen is completely overgrown by the antagonist (Fig. 9 D-F)

Effect of wheat bran based formulation of T. harzianum on growth of P. volubilis and suppression of leaf blight disease

Wheat bran based formulation as well as FYM based formulation, prepared from pure cultures of *T. harzianum* (Fig. 10) were amended on rhizosphere of plants and changes in the growth pattern of the plants were noted. Plant growth and number of leaves following application of *T. harzianum* has been presented in Table 3. Untreated plants artificially inoculated with *M. guilliermondii* showed leaf blight symptoms (Fig 11 A; Table 4). Plant growth of different stages following application of *T. harzianum* and challenge inoculation with *M. guilliermondii* have been documented in Fig 11 (B-F). Plant growth induced flowering at very early stages using FYM based formulation of *T. harzianum* (Fig 11 E). Induced resistance toward *M. guilliermondii* following treatment of biocontrol agent (*T. harzianum*, NAIMCC-F-01961) and induced flowering has been presented (Fig 11). Leaf blight symptoms was also reduced to some extent as evident with disease score and per cent leaf area affected (Table 4) after application of the formulation of *T. harzianum.*

DISCUSSION

The leaf blight symptoms appeared as circular necrotic sores on leaves. There was a dark brown border around the lesions. There were previous reports about incidence of leaf blight disease in cardamom (Bagchi *et al.* 2025), *Pseudostellaria heterophylla* (Zhang *et al.* 2025), rice (Behera *et al.* 2025). The causal fungal pathogen was identified as *Meyerozyma guilliermondii* (PQ467080) with spherical to sub spherical like cells with branched hyphae and cream-colored mature mycelium. Ghasemi *et al.* (2022) published a review article on *Meyerozyma-guilliermondii* species complex and its epidemiology and detailed mechanisms behind that. Jie lim*et al.* (2024) reported about the features of *Meyerozyma guilliermondii* as a rare fungal pathogen and discussed about its virulence factors. In phylogenetic analysis, *M. guilliermondii* (PQ467080) strain was found to be closely related with extype of *Meyerozyma guilliermondii* isolate mYJdd12 (KR632523). In favor of the above statement, previously by Vicente *et al.* (2025), genomic analysis of *Meyerozymaguilliermondii*CECT13190 was performed. Francisco *et al.* (2023) also reported the clades of *M. guilliermondii* species complex. Our M. guilliermondii isolate PV- 3 (PQ467080) has been tested and confirmed to be non human pathogenic strain. In support of this result, there were previous reports on hemolytic activity of fungal species (Tsang *et al.* 2007;Schaufuss and Steller 2003; Solgun *et al.* 2011; Theeb *et al.* 2013).

Through Koch's postulation, the leaf blight disease was reestablished and it was proved that the causal pathogen was *M. guilliermondii* (PQ467080). *Meyerozyma guilliermondii* was not only reported as foliar pathogen but its role as biocontrol agent was also found in some plants. In support of evidences regarding the role of *M. guilliermondii* as biocontrol agent, Robador *et al.* (2023) reported the role of *M. guilliermondii* in defense induction in grapevine plant against the pathogenic fungus *Fusarium equiseti*. Control of *Colletotrichum gloeosporioides* in mango plant was found by *M. caribbica*, as reported by Rosales *et al.* (2013). Balandrano *et al.* (2023) mentioned that *M. guilliermondii* and *M. caribbica* were used for the control of fungal pathogens

through the production of volatile compounds, hydrolytic enzymes and induction of disease resistance. Biocontrol potential of *Meyerozyma caribbica* YDP-27 in suppressing diseases by pathogens- *Colletotrichum musae, C. capsici, C. gloeos-porioides, Alternaria alternata, Curvularia alcornii* was reported by Baral *et al.* (2025). In our present study, *Meyerozyma guilliermondii* (PQ467080) was reported as first one as foliar pathogen of *Petrea volubilis*.

Trichoderma harzianum was used as potent biocontrol agent and in *in vitro* antagonistic tests, *T. harzianum* inhibited the growth of foliar pathogen *M. guilliermondii* with percent of inhibition of 83.5%. In favor of the biocontrol potential of *Trichoderma*, Yao *et al.* (2023) reported the role of *Trichoderma* in biocontrol and suppression of fungal diseases. There was report by Gveroska and Ziberoski (2012) about the antagonistic potential of *Trichoderma harzianum*. There were previous reports supporting the biological control of fungal diseases by *T. harzianum* (Hugar *et al.* 2025; Swathy *et al.* 2024; Povedo *et al.* 2024; Sood *et al*. 2020). In addition to the role of *T. harzianum*as biocontrol agent, *T. harzianum* was used in the form of wheat bran based as well as FYM based bioformulations for the improvement of growth of *Petrea volubilis*. Increase in height, number of leaves andinduced flowering were noted. Contreras-Cornejo *et al.* (2024) reported the role of *T. harzianum* as potent plant growth promoter and also discussed the detailed mechanisms behind the growth promotion. Role of *Trichoderma* formulations in growth promotion of plants was pointed out by Sudha *et al.* (2024). In another study by Allay and Chakraborty (2010), role of *T. hamatum* in inducing defense response in mandarin plants against root rot disease caused by *Fusarium* sp. was reported. Induced immunity developed by *Trichoderma* spp. in plants was presented by Chakraborty *et al.* (2020). Mukherjee (2023) also mentioned the health management of rice plants by *Trichoderma* strains. Khati and Chakraborty (2023) in their experimental study mentioned how *Trichoderma* spp. induced immunity in rice plant against the fungal pathogen *Dreschlera oryzae*. There were reports about the role of *Trichoderma harzianum* in enhancing the growth of plants (Wang *et al.* 2024; Sinha *et al.* 2024; Patel *et al.* 2024; Rigobelo *et al.* 2024). Leaf blight symptoms were also reduced after application of wheat bran based formulation of *T. harzianum*. Mollah and Hassan (2023) reported the ability of *Trichoderma harzianum* to reduce blight disease of potato. According to Pandey *et al.* (2024), up-regulation of the expression of pathogenesis related genes in reducing gray blight disease by *Trichoderma harzianum* strain TIND02. Zawawy *et al.* (2025) pointed out the biocontrol potential of *T. harzianum* against seedling blight disease caused by *Fusarium*.

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest

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