J.Mycopathol.Res. 63(1) : 187-195, 2024, ISSN : 0971-3719 (Print), 2583-6315 (Online) 10.57023/JMycR.63.1.2025.187

Molecular characterization and *in vitro* biocontrol of *Penicillium choerospondiatis* causing blue mold postharvest disease in Indian Gooseberry (*Phyllanthus emblica* L.) fruits in Manipur

KEISHAM NIRANJAN SINGH, KHUMBALLAMBAM ROSHIBINA DEVI, KISTU SINGH NONGTHOMBAM AND AMIT SETH*

Department of Life Sciences (Botany), Manipur University, Canchipur, Imphal, Manipur 795003

Received : 27.11.2024 Accepted : 31.01.2025 Published : 31.03.2025

Indian gooseberry fruits suffer heavy losses as postharvest products due to blue mold postharvest disease, resulting in rapid deterioration of fruit quality within no time, making it unfit for consumption. While keeping in mind the present scenario, the present study is undertaken to properly characterize the causal agent of blue mold disease of Indian gooseberry fruits, which remains utterly missing in the case of Manipur. The isolated pathogen from infected gooseberry has been confirmed as *Penicillium choerospondiatis* showing 99.82% similarity based on ITS-rDNA region sequencing. The pathogen also secretes amylase, cellulase and laccase hydrolytic enzymes. Moreover, *invitro* biocontrol activity while cocultured withtwo endophytic fungi has displayed class 3 antagonism as preliminary antifungal screening. Further assay revealed inhibitory percentage (I%) of 22.19% and 22.56% against *Fusarium solani* and *Chaetomium globosum*, respectively, which suggests the possible applicability of antagonistic fungi as biocontrol or disease management agents for the postharvest phytopathogenic fungi.

Keywords : Biocontrol, enzymes, Indian Gooseberry *Penicillium choerospondiatis*, Postharvest

INTRODUCTION

Fruits and vegetable become more susceptible to microbial contamination as postharvest products, with gradual progression of the ripening stage, which ignites more favorable microbial action (Sapadora and Dorby., 2016). In postharvest products, various physical damage occurs during the supply chain handling processes that can create wounds,serving as the infection initiation point for various microbes (Etefa *et al.* 2022). Approximately 25% of fruits and vegetables usually perish annually worldwide due to fungal contamination as harvested products (Petrasch *et al*. 2019), providing a necessary scope for studies related to coping with fungal contamination of fruits and vegetables.

Indian Gooseberry (*Phyllanthus emblica*L. or *Emblica officinalis*)is a vital fruit plant that is abundantly available and native to the Indian sub-continent and belongs to the family Euphorbiaceae (Koley and Nirala, 2020). Gooseberry is also profusely grown in North Eastern India, where the plant is included as an underutilized crop (Sengupta *et al.* 2020). In Manipur, Indian gooseberry fruits are also consumed in various forms, including fresh and dry fruits (Ayajuddin *et al.* 2016), as fruits have been reported for multiple health-beneficial compounds such as amino acids, polyphenols, antioxidant compounds, fruits are also noted for exceptional higher content of Vitamin C (Koley and Nirala, 2020). Despite the mentioned importance, fruit faces various shortcomings due to its highly perishable nature, as the harvested fruit faces repeat deterioration due to microbial infection. Bluemold disease is one of the significant infections that causes rapid deterioration within a short period and significant quality loss of the fruits (Koley and Nirala, 2020).

The quick degradation of postharvest crop productsis majorly contributed by the action of pathogenic microbes, of which various diverse

*Correspondence: amitsethshimla@gmail.com

fungal species have roles in fruit spoilage, in which mold infection is also a contributor that has caused repeated softening and degradation within a short period (Ngolong Ngea *et al.* 2021). Enzymes are functional proteins that various microor-ganisms produce (Pratush *et al*., 2017; Kumar *et al*.2015). The microbial enzymes are either intracellular (Bhatia *et al*.2017) or extracellular (Chauhan *et al*.2015, 2020). These microbial enzymes have diverse uses in industry (Singh *et al*., 2019, 2020) and agriculture (Chettri *et al*. 2021). The extracellular hydrolytic enzymes produced by the pathogenic microbes have a coordinated effect and aid in promoting the virulence nature of the pathogen, thereby leading to a repeated degradation of postharvest products (Ajayi *et al.* 2013; Al-Najada *et al.,* 2015). Primarily, enzymes secreted by pathogenic microbes can cause the degradation of fruit cell walls and have a role that enables the utilization of fruit cell wall materials as substrates causing cell degradation (Al-Hindi *et al.* 2011).

The fungal infection mitigating strategies in harvested products is one of the concerned areas of study relating to the use of safer management practices. Theuse of biocontrol agents (BCAs) is currently an emerging area of study, as these practices can replace hazardous chemicals which causes diverse harmful effects (Medeiros *et al.* 2012) as one of the BCAs, used of antagonistic microbes such as bacteria, yeast and fungi which can restrain the plant pathogenic microbes from spreading (Sharma *et al.*2017). BCA microbes express antagonistic effects through various mechanisms such as antibiosis (secretion of substances that are bioactive against pathogenic microbes),competition for space and nutrients (in which outcompeting pathogenic microbes for micro and macronutrient thus suppressing growth in postharvest products), induced resistance (in which BCAs such as application of antagonist induced expression of defense-related gene and enzymes such as PR protein)and parasitism (through co-culture in which active growth of antagonist suppresses pathogen growth as secretion of a lytic enzyme indicating contact inhibition) (Di Francesco*et al.* 2016).The present work focused on the isolation of the pathogen, characterisation, pathogenicity assessments, extracellular enzyme production, and *in vitro* biocontrol efficacy of the pathogen utilizing endophytic fungi.

MATERIALS AND METHODS

Survey and fruit sample collection

The survey was conducted at Imphal East, Manipur, where the Indian gooseberry plant was located at the specific site (24°48'31.5"N 93°58'51.4"E altitude 786.19 m), the herbarium was submitted at Manipur University Museum of Plant (MUMP) for proper authentication. In January 2024, gooseberry fruits with visible blue mold symptoms were collected.

Isolation of pathogen

The pathogen was isolated following the method of Kumari *et al.* (2021). The fruit sample was submerged in 70% ethanol for a duration of 1 min, followed by dipping in 4 % sodium hypochlorite for 3 min, again retreating in 70% ethanol for 15 s. Then, samples were washed thrice with sterilized distilled water and blotted dry. After drying, samples were cut into small pieces of about 5×5 mm^2, directly put into Potato Dextrose Agar (PDA) media supplemented with streptomycin to inhibit bacterial contamination, and incubated at 28±2 ^{0}C. The culture Petri plates were regularly monitored for detecting the emergence of fungal mycelia till complete spreading from day 2 to day 11; fungal hyphae were again transferred into a PDA plate and incubated for 5-7 days to ensure pure culture. Finally, emerged conidia weremoved into a PDA slant and preserved at -20^{0}C.

Morphological and molecular identification of Pathogen

Morphological identification of pathogen was done based on (Wang *et al.* 2017; Barnett and Hunter, 1998). Light microscopy (LM) based determ-ination of characters such as growth rate, colony color, the pattern of spore-bearing structure, and conidia shape were determined using LEICA750, and Scanning electron microscopy (SEM) of conidiophore was also taken using QUANTA 250.For molecular identification, DNA was extracted from fungal mats from freshly grown

Potato dextrose broth and genomic DNA isolation was performed based on the method described by Singh *et al.* (2024). ITS1, ITS2, and 5.8S rDNA region were amplified using universal fungal primer ITS4 (forward) and ITS5 (reverse). The PCR amplification reaction was performed with a reaction mixture of a total of 50 µl including a PCR master mix of 25 µl, DMSO of 2.5 µl, both forward and reverse primer of 1picomolar, and DNA sample of 100 ng/ µl.The PCR reaction was undertaken using a thermal cycler condition set at initial pre-denaturation at 94 ! for 5 min followed by denaturation of 30 cycles (1 min) at 94 ^{0}C, annealing reaction for 1 min at 50 ^{0}C, extension step for 1 min at 72^{0}C and final extension was performed for 7 min at 72^{0}C. The electrophoresis analysis of PCR product was conducted on 0.8% agarose gel. The amplified PCR product was sequenced on ABI-BigDye® Terminatorv3.1 Cycle Sequencing Kit (Applied Biosystems, Massachusetts, USA). Raw sequences obtained through SeqStudio Genetic Analyzer (Applied Biosystems, Massachusetts, USA) were edited manually for any inconsistency and the sequence was deposited in the National Center for Biotechnology Information (NCBI) to acquire GenBank accession number.

Phylogenetic analysis

Nucleotide sequences were compared with reference nucleotide sequences existing in the National Center for Biotechnology Information (NCBI) GenBank database, in which analysis was conducted using the Basic Local Alignment Search Tool (BLAST) to acquire the closest match. Phylogenetic tree construction is based on software Mega-X, version 10, utilizing the Maximum Likelihood method, and the clustered taxa have bootstrap test values of 1000 replicates (Saitou and Nei, 1987). The phylogenetic tree was constructed in which GenBank database sequences with more than 98% similarity with that of fungal pathogen sequences were used.

Pathogenicity test

To fulfill Koch's postulate and for confirmation of reisolated pathogen as a causal agent, a pathogenicity test was conducted following the methodof Gusella *et al.*(2020). The outer covering of fresh Indian gooseberry fruits was sterilized with 1.2% sodium hypochlorite solution for a time of 1 min, washed with sterile distilled water thrice, and blotted dry, after which fruits were wounded by punching with sterile cork borer in which fungal conidia from 7 days culture was inoculated and control fruits were kept without inoculation. All the inoculated and control fruits were placed in Petri plates separately, covered with sterilized plastic bags, and incubated for seven days at 25^{0}C.

Screening of fungal pathogen Penicillium chorespondiatis (PC01) for qualitative enzyme assay

The isolated *Penicillium chorespondiatis* (PC01) was tested for extracellular qualitative enzyme production following the method (Singh *et al.,* 2024); the assays include amylase, cellulase, lipase, and protease. The clear zone surrounding the fungal culture indicates the extracellular enzymatic activity resulting fromthe digestion of appropriate substrate supplemented in the agar media.

Amylase activity

Determination of amylase activity was conducted by inoculating the pathogenic fungus in the GYP agar media (glucose 1g; yeast extract 0.1g; peptone 0.5g; agar 16g) dissolved in 1000ml of distilled water) supplemented soluble starch 2%, then incubated for 3-5 days. The culture plates were subsequently flooded with 1% iodine in 2% potassium iodide solution to attain better clarity of halo formation. Finally, the appearance of clear halos around the culture was observed.

Cellulase activity

Determination of cellulase activity was performed by inoculating pathogenic fungus in a culture medium having 0.5% Carboxymethyl cellulose, that was supplemented in the GYP agar media (glucose 1g; yeast extract 0.1g; peptone 0.5g; agar 16g in 1000ml of distilled water). This culture was incubated at 28°C for a period of 3- 5 days. The culture plate was flooded with 0.1% Congo red. The dye was allowed to interact with the constituents of the petri plate for a period of 15 min. Subsequent destaining with 1M NaCl for 15

min was undertaken. Lastly, clear zones around the fungal culture were observed.

Laccase Activity

The capability to exhibit laccase activity was confirmed by inoculating the pathogenic fungus in GYP agar media (glucose 1g; yeast extract 0.1 g; peptone 0.5g; agar 16g) dissolving in 1000 ml distilled water supplemented with 0.005% 1-naphthol at pH 6 then incubated for 3-5 days. The observation of color changes of the transparent medium into blue or purple indicates the laccase oxidation of 1-naphthol.

Lipase activity

The capability to digest fat was determined by growing pathogenic fungus in peptone agar media (peptone 10g; NaCl 5g; $CaCl_2$ $2H_2O$ 0.1g; agar 16g) dissolving in 1000 ml distilled water) supplemented with tween 20 of 1% (v/v), then incubated for 3-5 days. The culture plate was finally observed for the appearance of a clear zone.

Protease activity

Determination of proteolytic activity by inoculating pathogenic fungus in GYP agar media (glucose 1g; yeast extract 0.1 g; peptone 0.5g; agar 16g) dissolving in 1000 ml distilled water supplemented with 1% skim milk, then incubated for 3-5 days and later observation of the culture plate for clear zone around the culture.

***In vitro* biocontrol activity using Petriplate Dual Culture Method**

Screening of antagonistic antifungal activity was tested against *Penicillium choerospondiatis* (PC01) using the endophytic fungi *Fusarium solani* (GenBank Accession No. OR357753) isolated from *Dichrocephala integrifolia* (Singh *et al*. 2024) and *Chaetomium globosum* (GenBank Accession No. OR397268) from *Chromolaena odorata* (Nongthombam and Mutum, 2023) by following the method described by Devi *et al.* (2022) in which pathogen and endophytic fungi were coculture in Petri plate containing PDA media inoculated 6 cm apart from each other and incubated at about 28±1 ^{0}C and recorded results at 7th and 10th days. The scale class score was implemented to determine the degree of antagonism against *Penicillium chaerospondiatis* where class 1 indicates complete replacement by endophytic fungi; class 2 indicates partial replacement by endophytic fungi covering at least two-thirds portion; class 3 indicates mutual inhibition exhibiting non-dominance over another covering only 50% of culture Petri plate; class 4, indicates partial replacement by pathogen covering at least two-third portion showing to withstand endophytic fungal activity and class 5, indicating complete replacement by pathogen exhibiting non-antagonist property. Moreover, further antifungal inhibition percentage (I%) was calculated following the methods of (Singh *et al.,* 2024) in which the formula I% = [(r1-r2)/ r1] × 100, where r1 is the radial growth diameter of the pathogen on the control plate, and r2 is the radial growth of the pathogen in coculture plate. Pathogen and antagonistic endophytic fungi were coculture in a Petri plate containing PDA media inoculated 6 cm apart and incubated at 28^{0}C for seven days.

RESULTS AND DISCUSSION

Survey and isolation of pathogen

Site survey of Indian gooseberries during the fruiting season displayed fruits with visible blue mold infection (Fig. 1 A), where fruits were covered extensively with blue mold infestation, giving off blue coloration and earthly smell. Mostly fruits lying in moist, shaded places have shown more visible infection symptoms,mainly fruits with abrasion created due to falling from trees, which signifies the importance of wounds in blue mold infection initiation (Janisiewicz*et al.* 2016). The correct identity of plant voucher/accession number 001381 was obtained for Indian Gooseberry (*Phyllanthus emblica* L.) after submission of the herbarium to Manipur University Museum of Plant (MUMP). The pure culture isolation of blue mold fungal pathogen in PDA culture media has shown complete radial growth at 11 days of incubation.

Morphological and molecular identification

As shown in (Fig. 1 B), the development of the pathogen in culture has exhibited a morphological dynamic appearance during the culture period. After the inoculation of the pathogen in the culture

Table 1: Qualitative extracellular enzyme activities of *Penicillium choerospondiatis* in which '+' signifies positive outcome and '-'signifies negative outcome.

Pathogenic fungus	Hydrolytic enzyme activities				
	Amylase	Cellulase	Laccase	Lipase	Protease
Penicillium choerospondiatis	+	+	+	-	-

Table 2: Represents the class of antagonism 7th and 10th day and Inhibition percentage (%) in which inhibitive is the mean data of triplicate ± standard deviation (SD) of endophytic fungi against *Penicillium choerospondiatis*.

Endophytic fungi	Class of antagonism in 7th day	Class of antagonism in 10th day	Inhibition percentage (I%)
Chaetomium globosum	Class 3	Class 3	22.56±0.16
Fusarium solani	Class 3	Class 3	22.19±0.23

media, the initial growth was observed within 1-3 days,indicated by the spread of fungal mycelia in a thin white hyphal mat, which continually turned yellowish and occasionally stained with an orange color. In the next 4-5 days, the central area of the culture started displaying a bluish-green color, and subsequently, the whole fungal culture turned bluish-green within the next 11 days of incubation with radial growth pattern, including micromorphological characteristics of fungal such as the presence of septate stipes, bi-verticillate conidiophores, presence of conidia in chains at phialides tip and ellipsoidal shape conidia (Fig. 1 C and 1 D). These features match with the generally identifying characteristic features of a fungus called *Penicillium choerospondiatis* (Wang *et al.*2017). The pure culture of the pathogenic fungus was deposited in a microbial culture depository (National Fungal Culture Collection of India, Pune)and granted an NFCCI deposition accession number of NFCC1 5737. Molecular identification of the fungal pathogen resulted in a 553 base pair long nucleotide sequence based on partial ITS-rDNA region sequencing, in which internal transcribed spacer (ITS) based region of nuclear ribosomal RNA (rRNA) operon is mainly accepted as a basic DNA barcode for a fungus sample and is subsequently assigned a species identity (Lucking *et al.* 2021). The BLAST alignment search resulted in maximum identity values with *Penicillium choerospondiatis* isolate PeGYF02 (OL967002.1) and *Penicillium choerospondiatis* isolate PeGYF01 (OL967001.1), both showing 99.82% similarity. Furthermore, the closeness with the species has also been confirmed with the construction of a phylogenetic tree (Fig. 2) based on the GenBank database sequence in which the GeneBank accession number of the isolated pathogen, i.e., *Penicillium choerospondiatis* isolate PC01 is PP869143.1. The isolated pathogen also shows close affinity with that of species reported from India as indicated by the phylogenetic tree in which highlighted isolated pathogens is in the same clades as that of species reported from India.

Pathogenicity test

Following pathogenicity test, the pathogen-inoculated fruits appear slightly water-soaked within 24h confined within the inoculation area (Fig. 3). As the infection spreads in the plant tissues, the inoculation area gradually turns bright yellow within 72 h and finally gives a bluish-green appearance and starts spreading, forming yellowish minute spots beyond the area of inoculation within 96h, in which the developmental pattern of blue also coincides with that of reported by (Koley and Nirala, 2020). The confirmed blue mold development and successful infectivity of the isolated pathogen followed Koch's postulate principles, also indicating fulfillment of Koch's postulate (Gusella *et al.* 2020).To successfully establishmold pathogens, the favorable conditions are the presence of wounded mature fruits, which are considered more susceptible, and an optimal temperature of 20-25^{0}C.Green mold conidial

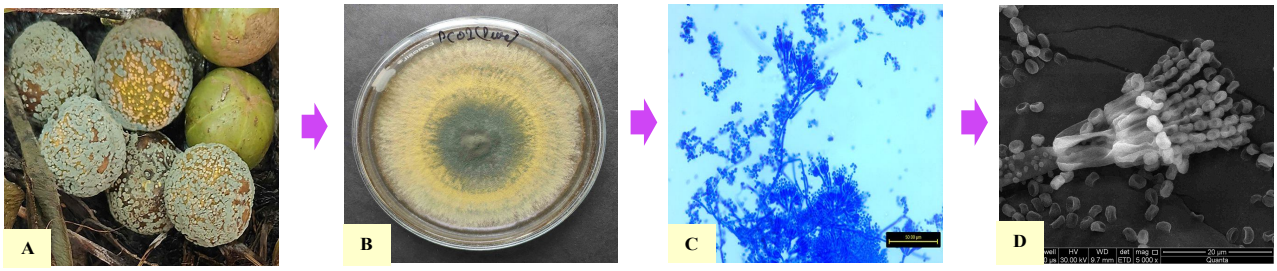

Fig 1: (A) Blue mold infected Indian gooseberry fruits, (B) Culture morphology of isolated *Penicillium choerospondiatis* and (C) Microscopic view of *Penicillium choerospondiatis* & (D) SEM image of *Penicillium choerospondiatis* conidiophore with chain of conidia

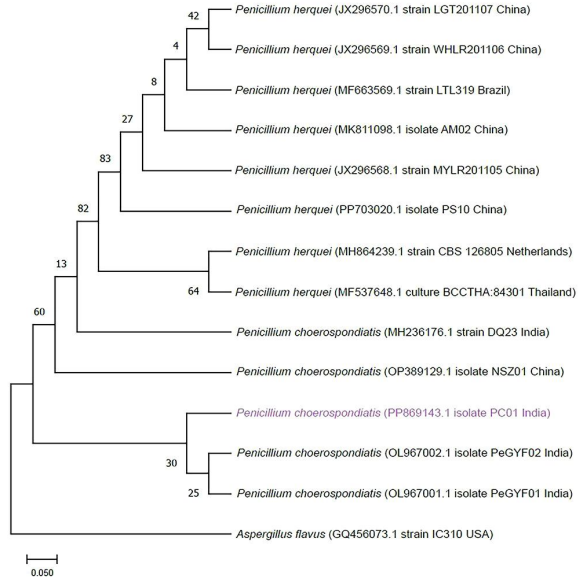

Fig 2 : Phylogenetic tree of *Penicillium choerospondiatis* (PP869143.1) with closely related species, constructed based on maximum likelihood method in which *Aspergillus flavus* (GQ456073.1) was taken as outgroup.

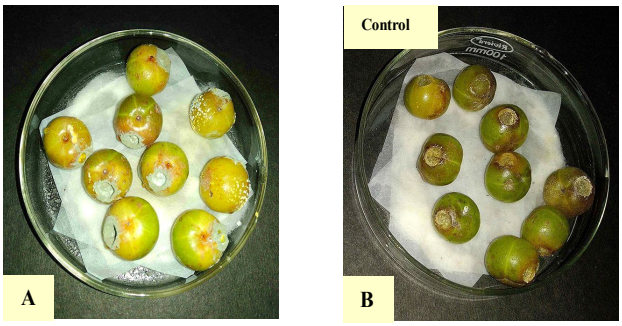

Fig 3: (A) *Penicillium choerospondiatis* inoculated fruits showing positive blue mold development and (B) Fruits without *Penicillium choerospondiatis* inoculation results with no evidence of disease development.

infection of fruits usually occurs at higher temperatures, while blue mold development is more rapid at lower temperatures (Papoutsis *et al,* 2019).

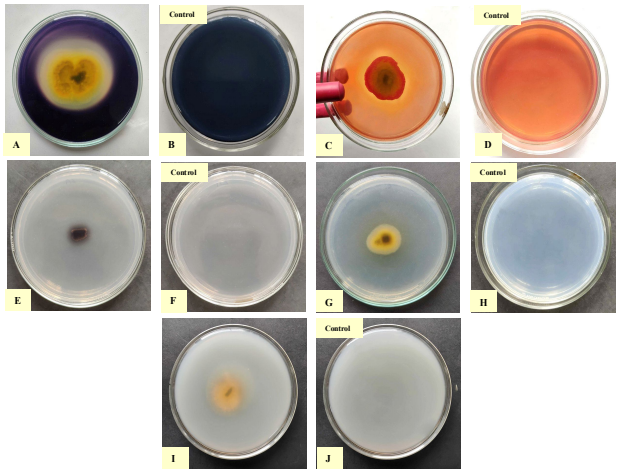

Fig 4: (A, C, and E) shows the positive extracellular amylase, cellulase, and laccase activity by *Penicillium choerospondiatis* as indicated by the development of halo region surrounding the culture, (G and I) indicates negative lipase and protease activity due to absence of halo region around the culture and (B, D, F, H and J) represents the control plates without fungal inoculation.

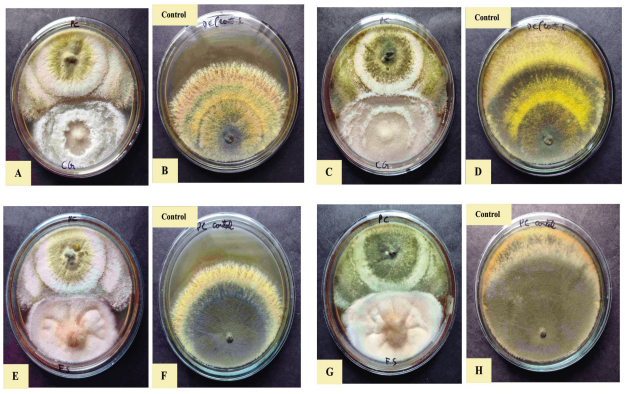

Fig 5: Represents the antagonistic effects of endophytic fungus against *Penicillium choerospondiatis*, in the co-culture plate phytopathogenic fungus is on the upper side, and endophytic fungi are on the lower side. (A and C) depicts the *Chaetomium globosum* antifungal activity against *Penicillium choerospondiatis* at 7th and 10th day of inoculation, (E and G) describes the *Fusarium solani* antifungal activity against *Penicillium choerospondiatis* within 7 and 10 days of inoculation, (B and F) represents the *Penicillium choerospondiatis* growth in control plate at 7th and (D and H) represents the *Penicillium choerospondiatis* growth in control plate at 10th day.

Penicillium choerospondiatis (PC01) qualitative enzyme production assay

The isolated fungal pathogen exhibited positive amylase, cellulase, and laccase activityby developing a visible halo zone, as seen in Fig. 4 A, C, and E whereas Fig. 4 B, D, and F represent the corresponding control plates. The results are also summarized in Table 1. Conversely, the pure culture of *Penicillium choerospondiatis* (PC01) did not exhibit lipase and protease enzyme activityas shown in Fig. 4G and I. The same observations are also represented in Table. 1**.** In the case of positive enzyme production activity, *Penicillium choerospondiatis* can solubilize supplemented substrate(1% iodine in 2% potassium iodide) thus clearly indicating the presence of amylase activity. Similarly, the development of a yellowish halo zone after the addition of 0.1% congo red and destaining with 1N NaCl around the culture indicates positive cellulase activity, and the presence of a bluish-purple halo zone around the culture from white color media after the incubation indicated a laccase activity due to oxidation of 1-naphthol). Various studies have reported the involvement of hydrolytic enzymes inestablishing pathogenesis in the host plant by fungi. They are vital for colonizing host plant tissue (Hussein *et al.*2020). Phytopathogenic fungi can secrete enzymes that can digest structural polysaccharides of host plant tissues, such as cellulose and amylose, in which cellulase and amylase are the enzymes that are important in the successful establishment of pathogenesis. These enzymes are included in classes of plant cell wall degrading enzymes($CWDE_S$) (Kaur *et al.* 2012). The enzyme laccase produced by fungi hasan extensive role in the depolymerization of lignin, which is one of the complex cell wall constituents in plant cells. The infectivity of pathogenic fungi displayed by fruiting body formation and sporulation isably supported by laccase production, thus supporting the repeat proliferation and penetration of pathogenic fungiwithin the host plant tissue. (Dwivedi *et al.*2011). Therefore, it may be deduced that producing various hydrolytic enzymes by blue mold fungus *Penicillium choerospondiatis* makes it a formidable agent of pathogenesis and the primary agent of blue mold postharvest disease of Indian gooseberry fruits. The fungal enzymes are the primary agents responsible for the repeated degradation of fruits within a few days just after the infection through the wounds, resulting in heavy losses of the fruits (Dutta *et al.,* 2023).

Invitro biocontrol using antagonistic endophytic fungi

Preliminary analysis of antifungal activity using co-culture for 7-10 days exhibited a class 3 degree of antagonism in which the endophytic fungi inhibited 50% of *Penicillium choerospondiatis* growth (Fig 5), indicating that the tested endophytic fungi hold an appreciable antagonistic effect. In addition, analysis of inhibitive percentage (1%) on the 7thday in which *Chaetomium globosum* and *Fusarium solani* shows 22.56±0.16% and 22.19±0.23% inhibitory percentage (Table. 2).The endophytic fungi have been thoroughly investigated as bio-potent agents capable of retarding phytopathogenic fungal growth (De Silva *et al.*2019;Armesto *et al.* 2020). The endophytic fungi express various suppressive mechanisms to out-compete phytopathogenic fungal growth (Adeleke *et al.* 2022). In the current study,gooseberry-damaging postharvest phytopathogenic fungi are limited in the expression of hydrolytic enzymes. In contrast, the endophytic fungi used as antagonistic agents are known to produce a wide array of hydrolytic enzymes, thus enabling them to successfully suppress the growth of blue mold fungus (Sopalunand Iamthama, 2022). The extracellular production of enzymes at elevated levels allows the endophytes to retard the growth progression of pathogenic fungi (Adeleke *et al*., 2022). The correct identification andcharacterization of phytopathogenic fungi of horticulturally important crops is a crucial and inevitable area and thus indicates their assimilation for the development of a feasible plant protection management practice that is green and ecologically compliant.

CONCLUSION

The present study demonstrated that *Penicillium choerospondiatis*is the phytopathogenic fungi of blue mold postharvest disease of Indian Gooseberry fruits in Manipur. This fungus's successful isolation and identification had not

been reported earlier in Manipur. Adequate morphological and molecular data support the identification of pathogenic fungi, and constructing a phylogenetic tree has indicated its lineage with known fungal species. Patho-genicity tests have confirmed the development of disease symptoms. Further characterization based on the production of hydrolytic enzymes has resulted in extracellular secretion of cell wall degrading enzymes (CWDEs) consisting of amylase, cellulase, and laccase activities, which can be correlated with its pathogenesis. *In vitro* biocontrol utilizing endophytic fungi indicated potential growth inhibitive capability of the endophytic fungi, indicating superiority against pathogenic fungal growth. The repressive action of endophytes against the targeted phytopathogen underlines their utility in the future development of a successful post-harvest disease management strategy.

ACKNOWLEDGEMENT

The authors thank Manipur University for providing a University fellowship to Keisham Niranjan Singh. In addition, the authors also thank Manipur University for providing the necessary laboratory facilities to carry out this research work.

DECLARATION

Conflict of Interest. The authors declare no conflict of interest.

REFERENCES

Adeleke, B. S., Ayilara, M. S., Akinola, S. A.,Babalola, O. O. 2022. Biocontrol mechanisms of endophytic fungi. *Egypt. J. Biologic. Pest Contr*. **32**: 46. https://doi.org/10.1186/s41938-022-00547-1

Ajayi, A. A., Osunlalu, E. O., Adejuwon, A. O. 2013. Studies on pectinolytic and proteolytic enzymes from deteriorated grapes (*Vitis vinifera*). *Covenant J. Physic. Life Sci.***1** https://journals.covenantuniversity.edu.ng/index.php/cjpls/article/view/92

Al-Hindi, R. R., Al-Najada, A. R., Mohamed, S. A. 2011. Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. *Afr.J. Microbiol. Res*. **5**: 443-448. https://doi.org/10.5897/AJMR10.896

Al-Najada, A. R., Gherbawy, Y. A. 2015. Molecular identification of spoilage fungi isolated from fruit and vegetables and their control with chitosan. *Food Biotechnol*. **29**: 166-184.https://doi.org/10.1080/08905436.2015.1027222

Armesto, C., Maia, F. G. M., Monteiro, F. P.,Abreu, M. S. D. 2020. Exoenzymes as a pathogenicity factor for *Colletotrichum gloeosporioides* associated with coffee plants. *Summa Phytopathologica* **45**: 368-373. https://doi.org/10.1590/0100-5405/191071

Ayajuddin, M., Modi, P., Achumi, B., Muralidhara, Yenisetti, S. C. 2016. Plant Products and Fermented Foods as Nutrition and Medicine in Manipur State of Northeast India: Pharmacological Authenticity. *Bioprospecting of Indigenous Bioresources of North-East India*. 165-179. https://doi.org/10.1007/978-981-10-0620-3_10

Barnett, H. L., Hunter, B. B. 1998. *Illustrated genera of imperfect fungi* (4th edition) American Phytopathological Society (APSPress), St. Paul.pp.218

Bhatia, K., Chauhan, K., Attri, C., Seth, A. 2017. Improving stability and reusability of *Rhodococcuspyridinivorans* NIT-36 nitrilase by whole cell immobilization using chitosan. *Inter. J.Biolog.Macromol*.**103**: 8-15. https://doi.org/10.1016/j.ijbiomac.2017.05.012

Chauhan, S., Jaiswal, V., Attri, C., & Seth, A. 2020. Random mutagenesis of thermophilic xylanase for enhanced stability and efficiency validated through molecular docking. *Recent Patents on Biotechnol*. **14**: 5-15. https://doi.org/10.2174/1872208313666190719152056

Chauhan, S., Seth, C. A., Seth, A. 2015. Bioprospecting thermophilic microorganisms from hot springs of western Himalayas for xylanase production and its statistical optimization by using response surface methodology. *J. Pure Appl.Microbiol*. **9**: 1417-1428.https://www.researchgate.net/publication/283819292

Chettri, D., Sharma, B., Verma, A. K., Verma, A. K. 2021. Significance of microbial enzyme activities in agriculture. In: *Microbiological Activity for Soil and Plant Health Management*(Eds. R.Soni D.C. Suyal, P. Bhargava, R.Goel). Springer, Singapore. 351-373.https://doi.org/10.1007/978-981-16-2922-8_15

De Silva, N. I., Brooks, S., Lumyong, S.,Hyde, K. D. 2019. Use of endophytes as biocontrol agents. *Fung. Biol. Rev*. **33**: 133-148. https://doi.org/10.1016/j.fbr.2018.10.001

Devi, W. S., Surendirakumar, K.,Singh, M. S. 2022. Distribution of endophytic fungi associated with Meriandra bengalensis Benth. and assessment of their bioactive potential in vitro. *Vegetos*. **35**: 995-1006. https://doi.org/10.1007/s42535-022-00374-7

Di Francesco, A., Martini, C., Mari, M. 2016. Biological control of postharvest diseases by microbial antagonists: how many mechanisms of action?. *Eur. J. Plant Pathol*. **145**: 711-717. https://doi.org/10.1007/s10658-016-0867-0

Dutta, M., Hazra, A., Bhattacharya, E., Bose, R., Mandal Biswas, S. 2023. Characterization and metabolomic profiling of two pigment producing fungi from infected fruits of Indian Gooseberry. *Arch.Microbiol*. **205**: 141. https://doi.org/10.1007/s00203-023-03483-2

Dwivedi, U. N., Singh, P., Pandey, V. P., Kumar, A. 2011. Structure–function relationship among bacterial, fungal and plant laccases. *J.Molecul. Catalysis B: Enzymatic*. **68**: 117-128. https://doi.org/10.1016/j.molcatb.2010.11.002

Etefa, O. F., Forsido, S. F., Kebede, M. T. 2022. Postharvest loss, causes, and handling practices of fruits and vegetables in Ethiopia: Scoping review. *J.Horticult. Res*. **30**: 1-10. https://doi.org/10.2478/johr-2022-0002

Gusella, G., Giambra, S., Conigliaro, G., Burruano, S., Polizzi, G. 2021. Botryosphaeriaceae species causing canker and dieback of English walnut (*Juglans regia*) in Italy. *Forest Pathol*. **51**: e12661. https://doi.org/10.1111/efp.12661

Hussein, M. A., Gherbawy, Y.,El-Dawy, E. G. 2020. Characterization, pathogenicity and enzymatic profile of *Fusarium solani* associated with potato tubers in Upper Egypt. *Arch. Phytopathology Plant Protect*. **53**:495-508. https://doi.org/10.1080/03235408.2020.1761223

Janisiewicz, W. J., Nichols, B., Bauchan, G., Chao, T. C., Jurick II, W. M. 2016. Wound responses of wild apples suggest multiple resistance mechanism against blue mold decay. *Postharvest Biol. Technol*. **117**: 132-140. https://doi.org/10.1016/j.postharvbio.2015.12.004

Kaur, S., Dhillon, G. S., Brar, S. K.,Chauhan, V. B. 2012. Carbohydrate degrading enzyme production by plant pathogenic mycelia and microsclerotia isolates of *Macrophominaphaseolina* through koji fermentation. *Industrial Crops and Products*. **36**: 140-148. https://doi.org/10.1016/j.indcrop.2011.08.020

Koley, C., Nirala, A. K. 2020. Detection, differentiation and mapping of different states of blue mold disease of Indian gooseberry (*Emblica officinalis* G.) using a biospeckle technique. *Eur. J. Plant Pathol*. **158**: 925-937. https://doi.org/10.1007/s10658-020-02127-1

Kumar, V., Seth, A., Kumari, V., Kumar, V., C Bhalla, T. 2015. Purification, characterization and in-silico analysis of nitrilase from *Gordonia terrae*. *Protein and Peptide Lett*. **22**: 52-62.http://dx.doi.org/10.2174/0929866521666140909154537

Kumari, P., Singh, A., Singh, D. K., Sharma, V. K., Kumar, J., Gupta, V. K., Bhattacharya, S.,Kharwar, R. N. 2021. Isolation and purification of bioactive metabolites from an endophytic fungus *Penicillium citrinum* of *Azadirachta indica*. *South Afr. J. Bot*. **139**: 449-457. https://doi.org/10.1016/j.sajb.2021.02.020

Lucking, R., Aime, M. C., Robbertse, B., Miller, A. N., Aoki, T., Ariyawansa, H. A., Cardinali, G., Crous, P. W., Druzhinina, I. S., Geiser, Da. M., Schoch, C. L et al. 2021. Fungal taxonomy and sequence-based nomenclature. *Nature Microbiol*. **6**: 540-548. https://doi.org/10.1038/s41564-021-00921-z

Medeiros, F. H. V. D., Martins, S. J., Zucchi, T. D., Melo, I. S. D., Batista, L. R., Machado, J. D. C. 2012. Biological control of mycotoxin-producing molds. *Ciência e Agrotecnologia*. **36**: 483-497. https://doi.org/10.1590/S1413-70542012000500001

NgolongNgea, G. L., Qian, X., Yang, Q., Dhanasekaran, S., Ianiri, G., Ballester, A. R., Xiaoyun Zhang, X., Castoria, R., Zhang, H. 2021. Securing fruit production: Opportunities from the elucidation of the molecular mechanisms of postharvest fungal infections. *Compr. Rev. Food Sci. Food Safety*. **20**: 2508-2533. https://doi.org/10.1111/1541-4337.12729

Nongthombam, K. S., Mutum, S. S. 2023. Biological activities and metabolite profiling of Chaetomium sp. R1C1, an endophytic fungus from *Chromolaena odorata* of Manipur. *Biologia* **79**: 643-656. https://doi.org/10.1007/s11756-023-01584-3

Papoutsis, K., Mathioudakis, M. M., Hasperué, J. H., Ziogas, V. 2019. Non-chemical treatments for preventing the postharvest fungal rotting of citrus caused by *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold). *Trends in Food Sci. Technol*. **86**: 479-491. https://doi.org/10.1016/j.tifs.2019.02.053

Petrasch, S., Silva, C. J., Mesquida-Pesci, S. D., Gallegos, K., Van Den Abeele, C., Papin, V., Fernandez-Acero, F.J., Knapp, S.J., Blanco-Ulate, B. 2019. Infection strategies deployed by Botrytis cinerea, *Fusarium acuminatum*, and *Rhizopus stolonifer* as a function of tomato fruit ripening stage. *Front. Plant Sci*. 10: https://doi.org/10.3389/fpls.2019.00223

Pratush, A., Seth, A.,Bhalla, T. C. 2017. Expression of nitrile hydratase gene of mutant 4D strain of *Rhodococcusrhodochrous* PA 34 in *Pichia pastoris*. *Biocatalysis and Biotransformation*. **35**: 19-26.https://doi.org/10.1080/10242422.2016.1247831

Sengupta, P., Sen, S., Mukherjee, K., Acharya, K. 2020. Postharvest diseases of Indian gooseberry and their management: a review. *Inter. J. Fruit Sci*. **20**: 178-190. https://doi.org/10.1080/15538362.2019.1608889

Sharma, M., Tarafdar, A., Ghosh, R., Gopalakrishanan, S. 2017. Biological control as a tool for eco-friendly management of plant pathogens.In: *Advances in Soil Microbiology: Recent Trends and Future Prospects: Volume 2: Soil-Microbe-Plant Interaction* (Eds. T.Adhya, B. Mishra, K. Annapurna, D. Verma, U. Kumar) 153-188. https://doi.org/10.1007/978-981-10-7380-9_8

Singh, N. K., Pandey, R. R., Singh, M. S. 2024. Biological activities and GC-MS analysis of crude extract of an endophytic fungus *Fusarium* sp. F1C1. *Vegetos*.37: 1720-1732. https://doi.org/10.1007/s42535-024-00817-3

Singh, P., Kumari, A., Attri, C., Seth, A. 2019. Efficient lactamide synthesis by fed-batch method using nitrile hydratase of *Rhodococcus pyridinivorans* NIT-36. *J. Microbiol. Biotechnol. Food Sci.***9**: 567-572.http://dx.doi.org/10.15414/jmbfs.2019/20.9.3.567-572

Singh, P., Kumari, A., Chauhan, K., Attri, C., Seth, A. 2020. Nitrile hydratase mediated green synthesis of lactamide by immobilizing *Rhodococcus pyridinivorans* NIT-36 cells on N, N2 -Methylene bis-acrylamide activated chitosan. *Inter. J. Biologic. Mol*. **161**: 168-176. https://doi.org/10.1016/j.ijbiomac.2020.06.004

Sopalun, K., Iamtham, S. 2020. Isolation and screening of extracellular enzymatic activity of endophytic fungi isolated from Thai orchids. *South Afr. J. Bot*. **134**: 273-279. https://doi.org/10.1016/j.sajb.2020.02.005

Spadaro, D., Droby, S. 2016. Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. *Trends in Food Sci. Technol*. **47**: 39-49. https://doi.org/10.1016/j.tifs.2015.11.003

Wang, X. C., Chen, K., Zeng, Z. Q., Zhuang, W. Y. 2017. Phylogeny and morphological analyses of *Penicillium* section Sclerotiora (Fungi) lead to the discovery of five new species. *Scientific Reports*. **7**: 8233. https://doi.org/10.1038/s41598-017-08697-1