AJAB

Asian J Agric & Biol. 2025(1).
DOI: 10.35495/ajab.2024.136

Original Article

Exploring mycoparasitic potential of indigenous *Trichoderma* strains for the effective control of red rot disease in sugarcane

Sana Riaz[1], Ghulam Mustafa[1*], Muhammad Sarwar Khan[1], Muhammad Amjad Abbas[2]
[1]Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan
[2]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

Received:
July 24, 2024
Accepted:
October 7, 2024
Published Online:
October 27, 2024

**Corresponding author email:*
drmustafa8@gmail.com

Abstract

Colletotrichum falcatum is the most infectious pathogen of sugarcane which not only affects crop productivity but also lowers sugar recovery. Vegetative mode of propagation, long breeding cycle and anatomy of the plant further vulnarize it to pathogen infection. Owing to limitations in the control of pathogen with chemicals and conventional methods, mycoparasitic agents are more effective and efficient. Indigenous *Trichoderma* strains were used in these studies to see their impact on the control of this infectious pathogen under *in vitro* and *in planta* conditions. Both, the pathogen and *Trichoderma* isolates were purified and confirmed through molecular tools. Phylogenetic analysis was carried out to assess their genetic relatedness with other *Colletotrichum* species. *In vitro* dual plate culture assay was performed to assess mycoparasitic potential of all of the isolates. *Trichoderma harzianum* showed 85.5% inhibition in the growth of *Colletotrichum falcatum* whereas *Trichoderma viride* showed 81.1% growth inhibition. Further, *in planta* infection assay was conducted in red rot susceptible sugarcane genotype SPF-234 to seek for its potential to suppress pathogen infestation. Six months old plants were inoculated with *C. falcatum* alone and in combination with *T. viride* by plug method of inoculation. The infected canes were dissected out and observed for red discoloration. Both of the biocontrol agents inhibited growth of the pathogen yet *T. harzianum* appeared to be more effective for the control of aforementioned pathogen. Hence, *Trichoderma harzianum* isolates can effectively be used for the control of red rot infection in sugarcane leading to enhanced crop production and sugar recovery.

Keywords: Red rot, Biocontrol agent, Sugarcane, Dual plate culture assay, *In planta* infection assay

How to cite this:

Riaz S, Mustafa G, Khan MS and Abbas MA. Exploring mycoparasitic potential of indigenous *Trichoderma* strains for the effective control of red rot disease in sugarcane. Asian J. Agric. Biol. 2025(1): 2024136. DOI: https://doi.org/10.35495/ajab.2024.136

Introduction

Sugarcane is the most significant cash crop cultivated for sugar and other by-products. It has a wide range of adaptability and is grown in more than 123 countries all over the World. Pakistan ranks 5th in area and 11th in production (Afghan et al., 2023). Various biotic and abiotic stresses are responsible for its low yield but the major cause of concern are diseases (Mehdi et al., 2024). The crop is infected by various diseases caused by bacterial, viral and fungal pathogens. Among fungal diseases, red rot referred as "cancer of sugarcane" is caused by *Colletotrichum falcatum* Went. (*Glomerella tucumanensis*) (Alexander and Viswanathan, 1996). In Pakistan, this disease was first reported in 1986 (Ahmed and Fasihi, 1986) and it may reduce cane production and sugar recovery by 29.07% and 30.8% respectively (Raza et al., 2023). The main reasons for disease infestation are vegetative mode of propagation, unavailability of disease resistant germplasm and effective chemical fungicides. Since chemical fungicides are the most common method used to control this infectious pathogen but not as effective as it should be (Haroon et al., 2023). In this context, biological agents are always preferred entities as they are human friendly and do not have any side effects likewise chemicals (Vandana et al., 2024). Naturally occurring antagonistic strains of bacteria and fungi have exclusively been worked out as biocontrol agents against a variety of pathogens. A number of research groups have worked out different accessions of *Trichoderma*, *Aspergillus*, *Penicillium* and *Chaetomium* etc. as potential antagonistic agents. Different suspensions have been developed for commercial scale application of these biocontrol agents including that of *Trichoderma*. Since *Trichoderma harzianum* is the most effective biocontrol agent against *Colletotrichum falcatum* (Ramzan et al., 2023). They produce hydrolytic enzymes i.e. chitinases, proteases, 1-3-glucanases which cause disruption of the fungal cell wall resulting in deterioration of the invading pathogens (Nadeem et al., 2022; Nadeem et al., 2023). In addition, they have ability to induce resistance in plants by the activation of secondary metabolites i.e. volatile antibiotics (6-pentyl-a-pyrone), water soluble compounds (heptelidic acid or koningic acid), peptaibols (linear oligopeptides of 15–22 amino acids) which help in pathogen degradation through symbiosis, differentiation and metal transport etc. (Misra and Ansari, 2021). Molecular biology has helped researchers in the characterization and screening of desired microbes which are of worth for humanity (Akhreim et al., 2024). Disease identification and remediation have been improved to great extent owing to the implication of scientific interventions. Likewise, developments in *in vitro* culture techniques have further helped it to develop realistic products (Costa et al., 2021).

We hypothesized that biocontrol agents (*Trichoderma harzianum* and *Trichoderma viride*) may prove an effective ecofriendly remedy for the control of red rot infection in sugarcane.

Keeping in view the significance of biocontrol agents for the eco-friendly management of sugarcane cancer (red rot), mycoparasitic potential of indigenous *Trichoderma* strains was explored in these studies. Further, *in planta* bioassay was also carried out to devise field based solution for the control of infectious pathogen.

Material and Methods

Isolation and purification of *C. falcatum* and *Trichoderma* isolates

The *C. falcatum* isolates were collected from red rot infected stalks of sugarcane from Faisalabad, TT-Singh, Bahawalpur, Jhang and Rahim Yar khan during the months of July and August (2020-2021). The samples collected were washed to remove dust particles. Further, sodium hypochlorite (NaOCl 1 %) was used for the surface sterilization of infected samples. The diseased samples were washed twice with sterilized distilled water after the application of sodium hypochlorite for one minute and placed on sterilized blotter paper to remove excess water. The infected segments (5 × 5 mm) were placed on potato dextrose agar (PDA) petri plates. The fungi were cultured and purified by single spore culture technique. The cultures were transferred to PDA slants, maintained at 4 ± 1 ºC until morphological characters were examined i.e color of mycelium (Purple type), spores shape (sickle/falcate) and size 28.7 × 4.1 µm (Figure 3). Simultaneously, two indigenous strains of *Trichoderma*: *T. harzianum* and *T. viride* were available in the laboratory (Mustafa et al., 2020). These strains had been characterized for the production of β-1,3-glucanase which is not only an important industrial enzyme but is also involved in delaying growth of pathogenic fungi.. These strains were cultured, maintained and incubated at 28 ± 1 ºC

for four days and purified on potato dextrose agar medium by single spore culture method (Figure 1).

Preparation of potato dextrose agar (PDA) media

Potato starch was extracted by boiling 250 g of unpeeled potato sliced in 250 mL of distilled water until half volume was attained and filtered through a washed muslin cloth to get potato fusion. For the preparation of 500 mL of PDA, 10 g of agar and 10 g of glucose were dissolved in distilled water and 150 mL of potato extract was added. Then, volume was made upto 500 mL and 500 ul of chloramphenicol (1 M rexaphenicol) was added at the time of pouring to avoid bacterial growth.

Molecular characterization of *C. falcatum* and *Trichoderma* isolates

Total genomic DNA was isolated from all of the fungal isolates of *C. falcatum*, *T. harzianum* and *T. viride* by using CTAB method with certain modifications. After DNA quantification, *C. falcatum* and *Trichoderma* isolates were characterized using internal transcribed spacer regions (ITS) primers specific for the identification of fungus. Sequence information was retrieved from (http://www.ncbi.nlm.nih.gov/) Genbank. The online Primer-X tool was used to design (ITS-1 5´-TCCGTAGGTGAACCTGCGG-3´ and ITS-4 3´-GCTGCGTTCTTCTTCATCGATGC-5´) primers. PCR reaction was carried out in total volume 20 µl containing 2 µl of *Taq* buffer, 3 µl of 10X, $MgCl_2$, 1 µl of 2.5 units of *Taq* polymerase, 0.2 µl of 10 M reverse and forward primers, 1 µl of 2 mM dNTPs and 2 µl of 100 ng/µl of genomic DNA. Thermal cycler profile of 30 cycles was followed: initial denaturation at 94 ^{o}C for 5 minutes, denaturation at 94 ^{o}C for 1 minute, annealing 52 ^{o}C and extension 72 ^{o}C for 1 minute respectively and final extension at 72 ^{o}C for 10 minutes. The resultant amplicons were resolved on agarose gel 0.8% (w/v) with 1 ul of 10 mg/ml ethidium bromide stock solution, to confirm amplification of the desired gene(s) (Mullis and Faloona, 1987).

***In vitro* antagonism by dual plate culture method**

All of the isolates of *C. falcatum* and *Trichoderma* were grown on PDA media and were incubated at 28 ± 1 °C (12 hr dark) for seven and four days respectively. *Trichoderma* strains (*T. harzianum* and *T. viride*) were tested for their mycoparasitic ability to control *C. falcatum* mycelium growth by dual-plate culture technique (Dennis and Webster, 1971). Both of the aforementioned fungi were co-cultured (inoculated at the edges of petri plates, one inch inside). Three replicates of each culture were investigated and incubated at 28 ± 1 °C. Growth pattern and colony diameter was observed from day 1 to 7 and growth rate was assessed. The fungal growth inhibition percentage was determined by the following formula (Watts et al., 1988).

$$\text{Percentage of growth inhibition} = \frac{R - R_1}{R} \times 100$$

R = Growth of the test pathogen (measured from the center of the colony towards center of the plate)

R1= Growth of the test pathogen (measured from the center of the colony towards antagonistic fungus)

***In planta* infection assay and data analysis**

Sugarcane plants of genotype SPF-234 were established in the pots. Six months old plants were inoculated during July-August (2022) using mixed culture of *C. falcatum* with *Trichoderma harzianum* (1:1) and *T. viride* (1:1) by the plug method of inoculation (Duttamajumder and Misra, 2004). In this regard, fungal cultures were multiplied on liquid filtrate PDB (potato dextrose broth) and incubated at 28 ± 1 ºC. Fungal cultures with 20-25 conidia per microscopic field were used to inoculate the internodal part of the plants and sealed with para film (Srinivasan and Bhat, 1961). Split-cane analysis was carried out after two months of inoculation in order to assess synergistic/antagonistic effects of *T. harzianum* and *T. viride* on *C. falcatum* infection.

Results

Purification and molecular characterization of *C. falcatum* and *Trichoderma* isolates

Molecular identification of the pathogenic agent *C. falcatum* and *Trichoderma* strains was carried out through PCR amplification of internal transcribed spacer regions (ITS). Amplification of a fragment of 550 bp from the genomic DNA of *C. falcatum* and amplification of 300 bp from the genomic DNA of *T. harzianum* and *T. viride* confirmed the said strains (Figure 2). The resultant amplicons were purified by the GeneJET PCR purification kit (Thermoscientific) and sequence characterized for further confirmation and validation. Phylogenetic analysis (Clustal-Omega) was carried out to assess genetic similarity of the pathogenic fungi with related accessions.

Figure-1. Purification of fungal pathogen and biocontrol agents on potato dextrose agar (PDA) (A) represents purification of *T. viride* (B) represents *T. harzianum* whereas (C) represents *Colletotrichum falcatum* (purple type)

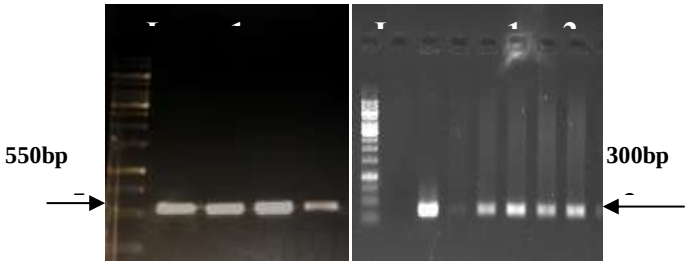

Figure-2. Confirmation of *C. falcatum* and biocontrol agents (*T. harzianum* and *T. viride*) by polymerase chain reaction using ITS primers. (A) Confirmation of *C. falcatum;* Lanes (1-4) indicate amplification from the genomic DNA of the said pathogenic agent (B) Lanes 1-3 represent amplification from *T. harzianum* isolates whereas Lanes 4-6 represent amplification from *T. viride* isolates. Lanes L represent 1 Kb DNA ladder.

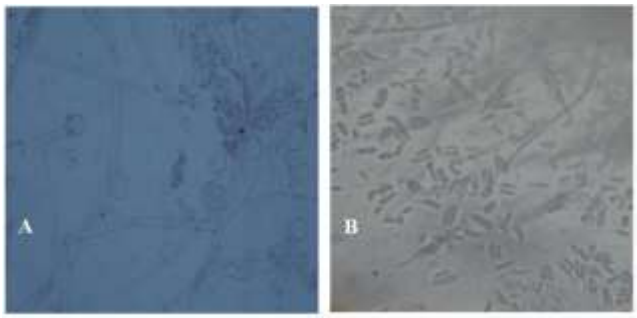

Figure-3. Morphological assessment of *Colletotrichum falcatum* isolates by microscopy. (A) Fungal mycelium with conidiophores, (B) Number and shape of conidia (28.7×4.1 µm, sickle shaped/falcate)

Phylogeny analysis

The sequences were assembled and edited by Clustal-omega run, to align the characterized sequences of *Colletotrichum falcatum* isolated from sugarcane in order to determine similarity index. Phylogenetic tree was constructed to find evolutionary relationship among *C. falcatum* isolate with other descendants of different species available in GenBank by using likelihood method (Figure 6). The analysis explored insights into the genetic relationships and evolutionary history of various *Colletotrichum* species, particularly highlighting the close genetic clustering within the *Colletotrichum falcatum* clade. The tree demonstrated that *C. falcatum* isolates share a high degree of genetic similarity, as evident from the high bootstrap values (1000 runs) showing a strong evolutionary lineage. *C. falcatum* appeared to be different from other *Colletotrichum* species, such as *C. higginsianum* (XM 018308313.1), *C. cereal* (EU 554182.1), and *C. eleusines* (EU 554234.1) indicating that these four species are genetically linked but diverse from each other.

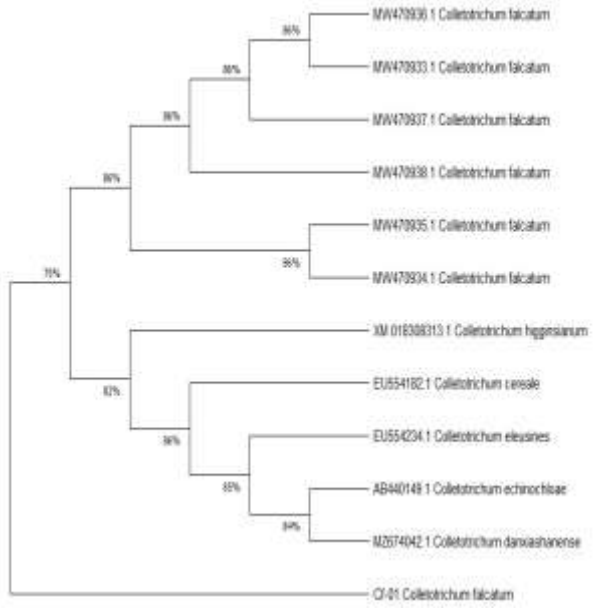

Figure-6. Neighbour-joining tree depicting genetic relatedness of *Colletotrichum falcatum* with *C .higginsianum, C. cereal, C. eleusines, C. echinochloae, C. danxiashanense* available in Genbank (NCBI) based on internal transcribed spacer sequences in which cluster appeared in a bootstrap test of 1,000 runs with percent frequency at nodes.

Assessing antagonistic effect of isolated mycoparasitic agents through dual plate culture assay

Mycelium growth of the pathogenic fungi (*Colletotrichum falcatum*) and mycoparasitic fungi (*T. harzianum* and *T. viride*) were observed on daily

basis after co-culture. Colony diameter of test pathogen *Colletotrichum falcatum* and both of the biocontrol agents were measured with the help of a scale. Both of the biocontrol agents appeared to arrest growth of red rot causal agent *C. falcatum* (Figure 7). Anyhow, *T. harzianum* appeared to be more effective as arrested growth of disease causing agent by 85.5% followed by *T. viride* which was able to arrest growth by 81.1% (Table 1). Hence, among the selected strains of the biocontrol agents, *T. harzianum* appeared to be more effective for the control of red rot causal agent *Colletotrichum falcatum* and can effectively be used to eradicate this disastrous pathogen.

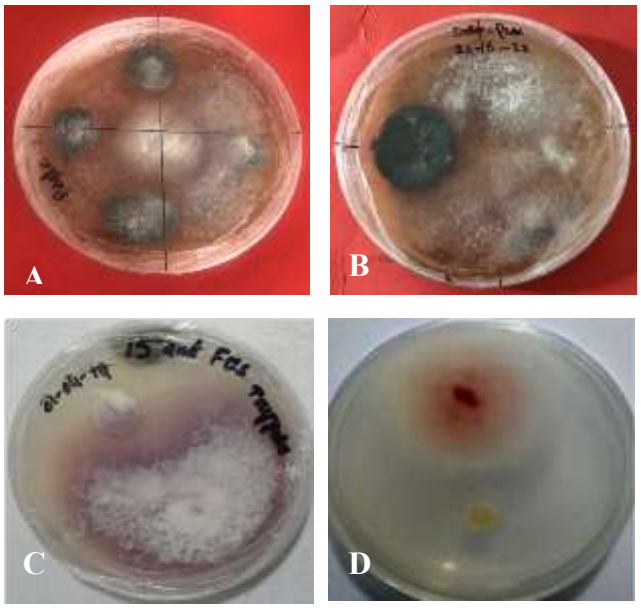

Figure-7. Dual plate culture assay showing mycoparasitic potential of the *T. harzianum* and *T. viride*. (A-B) represent antagonistic effect of *T. harzianum* whereas (C-D) represent antagonistic effect of *T. viride* on *C. falcatum*.

Table-1. Antagonistic effect of *Trichoderma* strains on the growth of *C. falcatum* under in vitro conditions

Sr No.	*Colletotrichum falcatum*	Diameter of colony (mm) *T. harzianum*	Inhibition (%) Growth	Diameter of colony (mm) *T. viride*	Inhibition (%) Growth
1.	P1	25	72.2	16.5	77.1
2.	P2	18.5	79	16	81.1
3.	P3	13	85.5	20.5	75.8
	Control	90		Control	85

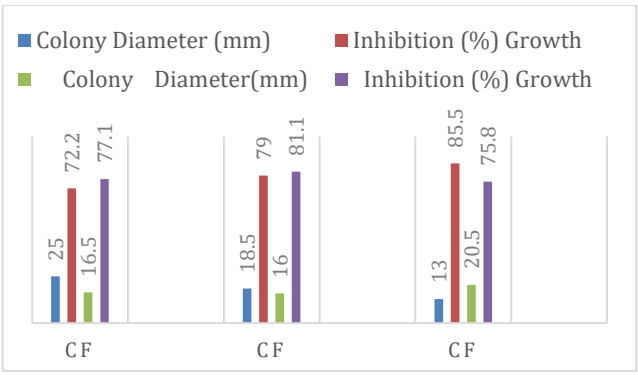

Figure-4. Comparative mycoparasitic effect of *T. harzianum* and *T. viride* against *C. falcatum* isolates. *Trichoderma harzianum* appeared to be more effective biocontrol agent with 85.5% growth inhibition as compared with *T. viride* which showed 81.1% inhibition in the growth of *C. falcatum*

***In planta* infection assay**

In planta infection assay was carried out to assess the effect of *Trichoderma* co-culture on *C. falcatum* in red rot susceptible sugarcane genotype SPF-234. The level of infection appeared to be decreased to great extent with both biocontrol agents i.e. *Trichoderma harzianum* and *Trichoderma viride*. Disease symptoms were observed after two months of inoculation when infected canes were dissected out and observed for red infection (Figure 5). Both of the biocontrol agents appeared to inhibit growth of the pathogen significantly whereas *Trichoderma harzianum* appeared to be better killer of the pathogen as compared with *Trichoderma viride*.

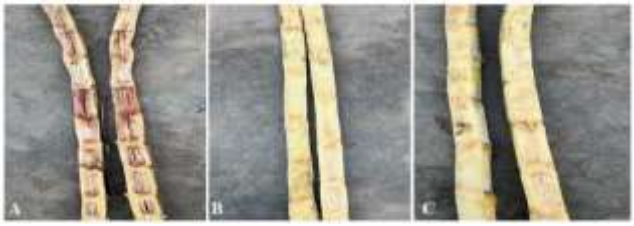

Figure-5. *In-planta* infection assay to assess mycoparasitic potential of the selected isolates of *T. harzianum* and *T. viride*. (A) represents *in planta* assay with *C. falcatum* whereas (B) represents inoculation with *C. falcatum* and *T. harzianum* (1:1 ratio). Likewise, (C) represents inoculation with *C. falcatum* and *T. viride* (1:1 ratio).

Discussion

Fungal pathogens are the most destructive agents responsible for reducing crop yield to a great extent. Red rot is the most noxious disease of sugarcane particularly in the sub-continent. It not only reduces crop yield but also affects sugar recovery and may even result in complete crop failure in case of severe disease attack. Chemical fungicides are the most famous method of disease control and Pakistan is importing pesticides (including fungicide) of worth 103 million USD annually (PACRA, 2022). These chemicals are not taken as desired entities owing to their side effects on human health. Biocontrol agents are of fundamental significance in this context as an alternative remedy for the control of fungal pathogens. Molecular biology has always helped mankind to explore novel molecules and characterize biocontrol agents. *Trichoderma* has proved to be the most valuable biocontrol agent against a wide range of pathogens and has been reported by numerous researchers as effective mycoparasitic agents (Mahmood et al., 2024; Mukhtar et al., 2021; Iqbal and Mukhtar, 2020) including the aforementioned pathogen of sugarcane. Owing to the significance of this biocontrol agent, the current studies were planned to assess effectiveness of *Trichoderma* for the control of *Colletotrichum falcatum.* Molecular characterization of both the pathogenic agent and biocontrol agents was carried out prior to assess mycoparasitic potential of *Trichoderma* for the control of *Colletotrichum falcatum.* Phylogenetic analysis of *Colletotrichum falcatum* retrieved ITS sequences revealed out its close relatedness with the already reported accessions available in Genbank *C. higginsianum, C. cereal, C. eleusines, C. echinochloae, C. danxiashanense* and *C. falcatum* based on internal transcribed sequences (ITS). It explored correlation between individual members of the *Colletotrichum* species by finding out their similarity matrix > 90 % which supports the earlier findings (Hossain et al., 2021; Costa et al., 2021; Viswanathan et al., 2020; Patel et al., 2018). Understanding these genetic relationships, particularly at intraspecific level is crucial to unravel the pathogenic mechanisms, host specificity and potential control strategies for fungal pathogens which are of significant importance for disease management (Shahid et al., 2023). Since, the study is unique because this pathogen has least been explored in our country, though numerous research groups have worked out this pathogen in India, Brazil, Bangladesh and Thailand (Viswanathan et al., 2022; Kashyap et al., 2021; Costa et al., 2021; Anuradha et al., 2020). No doubt, pathogen is infectious, its molecular characterization and phylogenetic analysis will help to further understand it and devise better strategies for its control (Aristya et al., 2024).

Further, we worked out biocontrol agents to assess their impact for the control of this pathogen and observed *Trichoderma harzianum* as an effective antagonist. The resultant data showed the lowest colony diameter and highest inhibition percentage of the test pathogen by *Trichoderma* strains. Inhibition of mycelial growth was recorded to be 85.5% for *C. falcatum* against *T. harzianum*. These findings are in-line with the research published by other researchers and can be of significant importance for the control of red rot disease in sugarcane. Iftikhar et al. (2023) reported the efficiency of *T. harzianum* and *T. viride* isolated from sugarcane rhizosphere and emphasized on the use of biological control agents as an alternative to chemical pesticides. Patil et al. (2023) screened out *Trichoderma astroviride* and *Trichoderma harzianum* against *Aspergillus niger, Colletotrichum falcatum, Curvularia lunata, Alternaria alternata* and reported that *Trichoderma* strains did not show variations in antagonism based on the rate of their hyperparasitism. In another study, inhibition potency of *Trichoderma* species such as *T. glaucum T. koningii, T. harzianum* and *T. viride* were studied to target this pathogen (Prince, 2015). *Trichoderma* strains exhibited inhibition potency against a variety of microorganisms by interrupting their activity (Malathi et al., 2008). Similarly, Singh et al. (2008) reported antifungal potential of two culture filtrates of *Trichoderma* species on woody fungi. Doi and Mori (1994) explored that the culture filtrates of various *T. harzianum* strains may be utilized to reduce the growth of red rot pathogen. Hence, seed treatment with *Trichoderma* cultures can lead to the establishment/accumulation of these biocontrol agents in the soil rhizosphere helping out to suppress the fungal pathogen infection (Iqbal and Muhtar, 2020). Additionally, *in planta* bioassay was carried out in red rot susceptible sugarcane genotype SPF-234 to evaluate the potential of *Trichoderma* strains for disease control. The data analysis revealed that plants inoculated with *C. falcatum* alone developed severe disease symptoms on leaves and stalks as compared to the plants inoculated with *C. falcatum* in combination with *Trichoderma* strains.

Hence *Trichoderma* isolates appeared to be effective biocontrol agents against *C. falcatum* and can be employed for the effective management of this noxious disease in sugarcane. Yet, there is a need to develop formulations of these *Trichoderma* strains for the better disease remediation at field level.

Conclusion

Exploiting ecofriendly solutions of the fungal pathogens is a need of the day. Molecular approaches are of pivotal importance in understanding these pathogens and potential biocontrol agents. The *Trichoderma* isolates under investigation were found to be potential biocontrol agents and can be employed for the control of fungal pathogen in sugarcane. *Trichoderma harzianum* is the most effective mycoparasitic agent as compared with *Trichoderma* viride, for the control of red rot infection in sugarcane. *Trichoderma harzianum* showed 85.5% whereas *Trichoderma viride* showed 81.1% inhibition in the growth of *Colletotrichum falcatum* in dual plate culture assay. Hence, dual plate culture assay and in planta assay revealed that the biocontrol agents have potential to control *Colletotrichum falcatum* infection in sugarcane hence a valuable alternative to chemical fungicides.

Acknowledgment

We are highly thankful to Higher Education Commission (HEC), Islamabad Pakistan for providing funds and Sugarcane Research Institute, AARI, Faisalabad Punjab Pakistan for providing germplasm.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: Higher Education Commission (HEC), NRPU Islamabad Pakistan.

Contribution of Authors

Riaz S: Conducted all of the experiments. The said research is part of her PhD thesis.
Mustafa G: Conceived idea, planned and supervised all research experiments, also managed funds for the said research work
Khan MS: Provided technical assistance, guidance and research facilities for the execution of this research.
Abbas MA: Provided technical guidance for fungal growth and preparation of co-cultures.

References

Aristya GR, Nabillah SAA, Kasiamdari RS, Damaiyani J and Prabowo H, 2024. Phylogenetic and genetic variation of sugarcane (*Saccharum* spp.) from Island of Java, Indonesia based on trnK chloroplast gene. Int. J. Agric. Biosci. 13(2):157-166.

Akhreim AA, Gaballa MF, Sulaiman G and Attitalla IH, 2024. Bio fertilizers production and climate changes on environmental prospective applications for some nanoparticles produced from some microbial isolates. Int. J. Agric. Biosci. 13(2):196- 203.

Afghan S, Khan ME, Arshad WR, Malik KB and Nikpay A, 2023. Economic importance and yield potential of sugarcane in Pakistan, In S. Afghan, M.E. Khan, W.R. Arshad, K.B. Malik and A. Nikpay (eds.), Sugarcane – Its Products and Sustainability. Intech Open.

Anuradha LK, Kumar R and Singh P, 2020. Cultural and morphological variability in isolates of *Colletotrichum falcatum* causal agent of red rot disease of sugarcane. Indian. J. Plant Prot. 48(2):74-84.

Alexander KC and Viswanathan R, 1996. Major diseases affecting sugarcane production in India and recent experiences in quarantine, pp. 46-48. In B.J. Croft, C.M. Peggin, E.S. Wallis and D.M. Hogarth, Sugarcane Germplasm Conservation and Exchange Report of an international workshop held in Brisbane, Queensland, Australia.

Ahmad MR and Fasihi S, 1986. Effect of different infection levels of red rot of sugarcane on cane weight and juice quality. J. Agric. Res. 24:129-131.

Costa MM, Silva BA, Moreira GM and Pfenning LH, 2021. *Colletotrichum falcatum* and *Fusarium* species induce symptoms of red rot in sugarcane in Brazil. Plant Pathol. 70(8):1807-1818.

Doi S and Mori M, 1994. Antifungal properties of metabolites produced by *Trichoderma* isolates from sawdust media of edible fungi against wood decay fungi. Mater. Organismen. 28:143-151.

Dennis C and Webster J, 1971. Antagonistic properties of species groups of *Trichoderma* II. Production of volatile antibiotics. Trans. Br. Mycol. Soc. 57(1):41-48.

Duttamajumder SK and Misra SC, 2004. Towards an ideal method of inoculation for screening sugarcane genotypes against red rot caused by *Colletotrichum falcatum*. Indian Phytopathol. 57(1):24-29.

Haroon M, Anas M, Naurin I, Afzal R, Irfan U, Tariq H, Idrees F, Taj MH and Rukh M, 2023. Autoimmunity in plants; a powerful weapon in kingdom plantae to combat stresses. Int. J. Agric. Biosci. 12(3):159-164.

Hossain MI, Ahmad K, Vadamalai G, Siddiqui Y, Saad N, Ahmed OH and Kutawa AB, 2021. Phylogenetic analysis and genetic diversity of *Colletotrichum falcatum* isolates causing sugarcane red rot disease in Bangladesh. Biology. 10(9):862.

Iftikhar R, Hafeez S, Masood S, Shaheen A and Manzoor M, 2023. Biological control of rice insect pest: a critical review. Agrobiol. Rec. 12:68-75.

Iqbal U and Mukhtar T, 2020. Evaluation of biocontrol potential of seven indigenous *Trichoderma* species against charcoal rot causing fungus, *Macrophomina phaseolina*. Gesunde Pflanzen, 72(2):195–202.

Kashyap L, Kumar, R and Singh P, 2021. Molecular and pathological characterization of isolates of *Colletotrichum falcatum* Went causing red rot in sugarcane. Agric. Res. J. 58(2):224-231.

Mahmood T, Adnan M, Moosa A, Mahmood M, Riaz M, Qammar S and Afzal M. 2024. Improved physiological responses and stress tolerance in cotton against *Fusarium* wilt with *Trichoderma harzianum* treatment. Plant Prot. 8(2):217-228.

Mehdi F, Cao Z, Zhang S, Gan Y, Cai W, Peng L, Wu Y, Wang W and Yang B, 2024. Factors affecting the production of sugarcane yield and sucrose accumulation: suggested potential biological solutions. Front. Plant Sci. 15:1374228.

Misra V and Ansari MI, 2021. Role of *Trichoderma* in agriculture and disease management, pp. 425-440. In H.I. Mohamed, H.ED.S. El-Beltagi, K.A. Abd-Elsalam (eds.), Plant Growth-Promoting Microbes for Sustainable Biotic and Abiotic Stress Management. Springer, Cham, Switzerland.

Mukhtar T, Tariq-Khan M and Aslam MN. 2021. Bioefficacy of *Trichoderma* species against javanese root-knot nematode, *Meloidogyne javanica*, in green gram." Gesunde Pflanzen. 73(3):265-272.

Mustafa G, Anwar S, Joyia FA, Hayat MB, Zia MA and Khan MS, 2020. Molecular characterization and mycoparasitic aptitude of indigenous biocontrol agent *Trichoderma harzianum*. J. Anim. Plant Sci. 30(6):1508-1515.

Malathi P, Padmanaban P, Viswanathan R and Mohanraj D, 2008. Interaction between *Colletotrichum falcatum* pathotypes and biocontrol agents. Arch. Phytopathol. Plant Prot. 41(5):311-317.

Mullis KB and Faloona FA, 1987. Specific synthesis of DNA *In vitro* via a polymerase-catalyzed chain reaction. Methods Enzymol. 155:335-350.

Nadeem M, Khan AA, Khan AA, Nadeem J and Fatima U, 2023. Cloning and characterization of *Trichoderma* glucanase gene for plant transformation. Int. J. Agric. Biosci. 12(1):31-46.

Nadeem M, Ahmad B and Shaukat F, 2022. Elucidate the role of glucanase gene against fungal pathogens. Agrobiol. Rec. 10:26-34.

Patil TV, Pangrikar PP and Mane SB, 2023. Studies on antagonistic activity of *Trichoderma* against fungi. Int. J. Eng. Sci. Math. 12(6):47-54.

PACRA (The Pakistan Credit Rating Agency Limited), 2022. Pesticides Sector Study. Pakistan.

Patel P, Rajkumar BK, Parmar P, Shah R and Krishnamurthy R, 2018. Assessment of genetic diversity in *Colletotrichum falcatum* Went accessions based on RAPD and ISSR markers. J. Genet. Eng. Biotechnol. 16(1):153-159.

Prince L, 2015. Screening and characterized the antagonistic fungus of *Trichoderma koningii* against *Colletotrichum falcatum* by food poisoning technique. Glob. J. Res. Anal. 4(2):153-156.

Ramzan U, Abid K, Zafar MA, Anwar AM, Nadeem M, Tanveer U and Fatima U, 2023. *Trichoderma*: multitalented biocontrol agent. Int. J. Agric. Biosci. 12(2):77-82.

Raza HA, Hameed MU, Islam MS, Lone NA, Raza MA and Sabagh AEL, 2023. Environmental and Economic Benefits of Sustainable Sugarcane Initiative and Production Constraints in Pakistan: A Review, pp. 441-468. In M. Ahmed (eds.), Global Agricultural Production: Resilience to Climate Change. Springer, Cham, Switzerland.

Shahid A, Faizan M and Raza MA, 2023. Potential role of silver nanoparticles (AgNPs) and zinc nanoparticles (ZnNPs) for plant disease management. Agrobiol. Rec. 14:59-69.

Singh V, Joshi BB, Awasthi SK and Srivastava SN, 2008. Ecofriendly management of red rot disease of sugarcane with *Trichoderma* strains. Sugar

Tech. 10:158-161.

Srinivasan KV and Bhat NR, 1961. Red rot of sugarcane: Criteria for grading resistance. J. Indian Bot. Soc. 40:566-577.

Vandana P, Singh D, Srivastava S and Guru GR, 2024. Suppression of sugarcane red rot disease through its rhizospheric mycoflora. J. Exp. Agric. Int. 46(5):98-109.

Viswanathan R, Singh SP, Selvakumar R, Singh D, Bharti YP, Chhabra ML and Minnatullah M, 2022. Varietal break down to red rot in the sugarcane variety Co 0238 mimics Vertifolia effect: characterizing new *Colletotrichum falcatum* pathotype CF13. Sugar Tech. 24(5):1479-1496.

Viswanathan R, Padmanaban P and Selvakumar R, 2020. Emergence of new pathogenic variants in *Colletotrichum falcatum* stalk infecting ascomycete in sugarcane: role of host varieties. Sugar Tech. 22(3):473-484.

Watts R, Dahiya J, Chaudhary K and Tauro P, 1988. Isolation and characterization of a new antifungal metabolite of *Trichoderma reesei*. Plant Soil. 107:81-84.