Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2024i1.1316
*Biol. Clin. Sci. Res. J., Volume, **2024**: 1316*
Original Research Article

EFFECTIVENESS OF *TRICHODERMA* SPECIES IN THE MANAGEMENT OF SUGARCANE WILT (*FUSARIUM SACCHARI*)

FATIMA A[1], KHAN YSA[2*], IQRA[3], TALHA A[4], JAN A[2], ASAD Z[5], SABA H[6], ASLAM AH[2], BATOOL M[1], ASHRAF ZU[2]

[1] *Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*
[2] *Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan*
[3] *Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan*
[4] *Institute of Plant Protection, Muhammad Nawaz Shreef University of Agriculture, Multan, Pakistan*
[5] *Center for Agriculture and Bioscience International, Pakistan*
[6] *Department of Environmental Sciences, Government College University Lahore, Pakistan*
Corresponding author email address: gmustafaly@gmail.com

(Received, 10nd August 2024, Revised 26th October 2024, Published 29th November 2024)

Abstract *Sugarcane is a major cash crop in Pakistan. It has a great contribution in world's economy. Many diseases cause many losses in yield of sugarcane. Fungi are considered as abundant traitorous pathogens that obstruct the viable growth of sugarcane crop. Wilt disease caused by fungal pathogen Fusarium sacchari caused many losses in sugarcane production in Pakistan. Fusarium sacchari is a causative fungus of wilt in sugarcane. Biocontrol agent Trichoderma spp. used in the management of sugarcane wilt. These bio-agents help in diseases management and play important role in increasing the crop yield. For this reason, these bio-agents are used for biological maintaenace of crop diseases and research globally. The use of PDA medium in vitro revealed antagonistic activity of various Trichoderma isolates against the sugarcane wilt pathogen Pathogenicity test also executed to check the most aggressiveness strain of Fusarium spp. to cause disease in sugarcane plant. In vivo, Trichoderma multiply on different substrates and corn Cobbs is good byproduct Trichoderma multiply on it and mix in soil of pots. Pot experiment performed with different treatments to control wilt disease by using of antagonist's to check disease incidence and disease reduction.*

Keywords*:* *Sugarcane; Wilt; Fusarium sacchari; Biocontrol; Trichoderma species*

Introduction

Fungi are important in ecosystems as endophytes, pathogens, and saprobes (Bhunjun et al., 2022). Many pathogens, including viruses, fungus, and bacteria, attack on the crop during its 12-18 months after germination, resulting in the establishment of 200 different diseases. (Karamchandani et al., 2022). Diseases instigated by various pathogens including fungi pose severe threat to sugarcane crops (Viswanathan et al., 2018). Fungi are classified as liberal traitorous pathogens that inhibit the viable growth of the sugarcane crop. (Karamchandani et al., 2022). Fungi can cause most severe diseases in sugarcane crop (Diao et al. 2017). Fungi are responsible for more than 60 diseases in sugarcane (Mehnaz, 2013). More than 60 sugarcane diseases have been linked to various fungus (Mehnaz, 2013). Wilt, also known as alternate stalk disease, has a significant impact on sugarcane productivity and is caused by Fusarium sacchari. Fusarium sacchari is the cause of wilt in sugarcane (Poongothai, Viswanathan, Malathi, & Sundar, 2014). Wilt is a known to be a serious disease of sugarcane that can infect the crops at all stages from germination to maturity stage. (Viswanathan 2020). It mainly reduced germination percentages in the said crop (Viswanathan & Rao, 2011). Yield losses due to pathogen generally reported by 19 to 20% (Singh RS, 2018). The curve of macroconidia of *Fusarium* species can be disseminated by rain. Furthermore, the condio-spores are simple and branched produced single celled hyaline oval to elongated micro conidia and the fungal mycelium is septate and thin walled. (Deacon, 2006).

Characteristic symptoms of wilt are yellowing and wilting of leaves, reduced growth, and red coloration of the xylem vessels (Okungbowa & Shittu, 2012). *Fusarium sacchari* is the root/stalk pathogen that is infect the roots and stalks cause foliar infection. Additionally, *Fusarium sacchari* infections serve as the main source of damage and it remain as it is in the canes for months before appearance of diseases

[Citation: Fatima, A., Khan, Y.S.A., Iqra, Talha, A., Jan, A., Asad, Z., Saba, H., Aslam, A.H., Batool, M., Ashraf, Z.U. (2024). Effectiveness of *Trichoderma* species in the management of sugarcane wilt (*Fusarium sacchari*). *Biol. Clin. Sci. Res. J.*, **2024***: 1316*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1316]

symptoms depending on prevailing weather and crop stage. (Lockhart et al., 2000).

The use of fungicides has provided many benefits i.e. excessive use of fungicides threatens human animal life and environment. Fungicide use has also not produced desirable outcomes for wilt management, and it is hazardous to the human health and environment (Jahanshir and Dzhalilov, 2010). In this case, bio-control agents such as Trichoderma spp. provide a viable and environmentally friendly choice for disease management. Trichoderma spp. are soil-inhabiting fungus that have been employed to manage plant diseases. Prasad and Rangeshgwan, (2000) reported their bio efficacy. When *Trichoderma spp.* enter the pathogen-produced enzymes to controlling the soil borne pathogen this property of *Trichoderma spp.* to be very useful. In other crops these fungi are proved to be useful in disease managment (Sharma et al., 2014: Irfan et al., 2024). *Trichoderma spp.* as a potent fungal biocontrol for a variety of plant pathogens (Rini and Sulochana, 2008).

Sugarcane (Saccharum Officinerum L.) being cash crop, provides income as well as employment to the farming population (Ahmad et al., 2022: Abbas et al., 2024ab; Rehman et al., 2024; Sami et al., 2023). The demand of sugar and corn fructose is increasing day by day in globally. Pakistan discovered sugarcane production (12.8 million tons), and it is now the world's 6th largest producer (Khan, M. E. 2022). Sugarcane is used to make sugar and biofuels, among other things. Sugarcane used in many industries producing many products like alcohol, beverages, and bagasse used for paper, ethanol used for fuel, press mud and the production of chipboard. Sugarcane is the primary source of sucrose, accounting for over 80% of global sugar production (FAO. FAOSTAT 2022). Sugarcane is an excellent source of organic matter and nutrients for crop production (Bailey, 2004). According to a recent report published by the USDA (USDA, 2021-2022), sugarcane production was predicted to be decreased due to offending monsoon scenarios (floods and droughts) and pathogen attacks (Karamchandani et al., 2022). The current study was designed to evaluate a suitable number of endophytic and rhizospheric species of Trichoderma and their isolates against the sugarcane wilt casual organism i.e. *F. sacchari*.

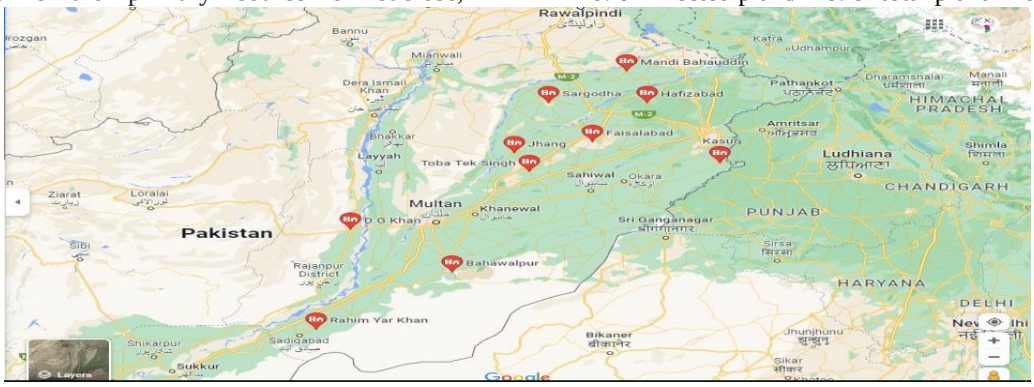
Material and methods

Surveillance of different sugarcane growing areas of Punjab

Survey conducted to collect different sugarcane diseased samples and soil samples from following 10 districts of punjab (Sargodha**,** D.G. Khan, Rahim Yar Khan, Jhang, Faisalabad, Hafiz Abad, Kasur, M.B.Din. Toba Tek Singh, Muzaffargarh) (table 1). The sampling locations were indicated on maps with google maps (Figure 1). To investigate the destruction of disease (leaf withering, yellowing, reduced growth, and red staining of xylem vessels), 20 farms and sugar mill fields in each area were investigated. Randomly take 25 samples (containing of stalks and leaves) having disease symptoms of each field from different districts of Punjab by z-scheme method according to already renowned protocols (Teng, 1983). Labelling were propered. Percentage disease incidence (PDI) for said disease will be estimated according to Singh et al. (2012) as follows:

Percentage Disease incidence =

No. of infected plant/ No. of total plant ×100



Map Figure 1: Sampling spots of different districts of Punjab

Isolation, morphological and molecular identification of fungus

Sample that showed wilt symptoms were collected from following districts and transfered to mycology laboratory of Crop Diseases Research Institute NARC for the characterization of the pathogen (figure 3). Sugarcane samples washed with distilled water, dried and cut the stems vertically. Take 2mm disc of diseased sample dipped into 10% sodium hypochlorite for 2min, place the sterilized sample of sugarcane on PDA plates, and kept at 28C for 5-7 days in incubator. Fresh mycelium of pathogenic fungi were transferred through hyphal tip method into PDA media slants and preserved as stock cultures for further analyses (figure 4). Separation of antagonistic fungi were completed through serial

[Citation: Fatima, A., Khan, Y.S.A., Iqra, Talha, A., Jan, A., Asad, Z., Saba, H., Aslam, A.H., Batool, M., Ashraf, Z.U. (2024). Effectiveness of *Trichoderma* species in the management of sugarcane wilt (*Fusarium sacchari*). *Biol. Clin. Sci. Res. J.*, **2024***: 1316*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1316]

dilution method on PDA plates and kept at 28℃ for 5-6 days in incubator. Spores were Identified through compound microscope (Elad and Chet, 1983). All these organisms were preserved on PDA slants at 4℃ for future use. Mycelium were collected from both pathogenic and antagonistic fungi from 7-day-old PDA plates, and their DNA was extracted by CTAB method (Rukmana et al., 2020) (figure 5).

In vitro effect of antagonistic fungi against pathogenic fungi

The antagonistic effect of biocontrol fungus on pathogenic fungi was assessed using the dual culture approach. Pathogen mycelial plugs measuring 5mm were put on one side of a 90mm PDA plate with antagonist fungal isolates. Plates were placed on another side and incubated at 28℃ in an incubator. The growth of test pathogens in control and treated pathogens was recorded after 5-7 days, and the percentage inhibition of pathogen mycelia growth was estimated using the formula below. I (%) = (C-T/C) * 100

Where I is the percent inhibition of the pathogen; C is the colony diameter in control; and T is the colony diameter after treatment. (gawade et al., 2012).

Pathogenicity test

Sugarcane seedlings were inoculated with F. sacchari strains (AA, TH-10, KC8, F.S, S4, M4, and S3). Each isolate was grown on 9-cm Petri plates with PDA and stored at 25°C in the dark for a week. The inoculum was prepared in water and adjusted to 2×106 conidia per ml^{-1} using a haemo-cytometer. Sugarcane setts were sterilized with 0.26% sodium hypochlorite for 30 minutes, positive controls were steeped in 500 mL of spore suspension at a concentration of 2x106, and negative controls were soaked in water for 12 hours before being transplanted into a pot. Nordahliawate, Izzati, Azmi, and Salleh (2008).

Treatments were systematized in a CRD with three replications. Disease symptoms were assessed 15 and 30 after inoculation (day), the symptoms were observed using a descriptive scale proposed by (Nordahliawate et al., 2008) Koch's postulates were accomplished by re-isolation of the pathogens from diseased plant tissue.

Pot experiment

To examine the biocontrol effect of antagonist fungi, specifically un-1, N.F, and G.M, on four isolates of Fusarium wilt of sugarcane in a greenhouse. Soil (including silt, sand, and decomposed FYM in equal proportions, i.e., 1:1:1) was produced and autoclaved for 1 hour on two consecutive days. Soil was modified with fungal antagonists cultivated on sorghum grains, and 10ml and 20ml broths of antagonist fungi and pathogens were added to individual soil pots containing 5kg dirt. Thirty-day-old seedlings were used to be transplanted into plastic trays holding soil at a pace of three pots per treatment.

To check the efficacy of antagonistic fungus against sugarcane wilt with 5 treatments of two concentration (5g/1kg soil; 10g/1kg soil). In treatment 1 pathogenic fungi (25g con1/50g con2) inoculate in soil and mix soil properly. After one week of inoculation the seedling of sugarcane dip into the antagonistic fungi (2ml/4ml) suspension and growing into pots and check the effect on seedling of sugarcane. In treatment 2 first we inoculate the (25g conc1/ 50g conc2) in the soil and mix soil properly.

After one week of inoculation the seedling of sugar cane dip into pathogenic fungi (10ml/20ml) suspension and growing the seedling into pots and check the effect on seedling of sugarcane. In treatment 3 both antagonistic fungi and pathogenic fungi (25g con1/ 50g con2) inoculate at the same time and check the effect on seedling of sugar cane. In treatment, 4 only pathogenic fungi (25g conc1/50g conc2) apply on plants. In treatment, 5 only antagonistic fungi (25g conc1/30g conc2) apply on plants and check the effect on seedling of sugarcane. Treatment 6 is negative control only applied water these are control plants. Readings were assessed after 15, 30, 90,120 and 160 days.

Result

Surveillance of different sugarcane growing areas of Punjab:

The sugarcane wilt incidence rate across all discussed districts was ranging between 10.50% and 60.59%. The Jhang district exhibited maximum wilt incidence (60.59%), followed by D.G khan 40.4% and Sargodha (28.5%). The lowest wilt incidence (10.50%) was recorded in Kasur districts (figure 2).

Identification of *Fusarium sacchari*:

All strains under study were identified as *F. sacchari* based on their physical appearance. On PDA, the development was rapid, with a profusion of mycelia ranging from colorless to pale violet and brownish violet. The pigments ranged from colorless to grayish orange, turning purple with age. After 3 days, colonies grew to a diameter of 2.3-3.70 cm at 25°C and 2.1-3.40 cm at 30°C. The conidiophores were primarily branched at one level. Macro conidia are thin, slender, slightly falcate, thin-walled, 3-4 septate, and range in size from 19 - 46.2 x 2.70 - 3.31 µm. The micro conidia were oval and somewhat 0-1 septate, measuring 5.41 - 15 x 1.91 - 4.10 µm.

Identification of antagonistic fungi

Typical fungal hyphea grows up to a diameter of 10 µm. Asexual spore formation produces single-celled, green conidia (3 to 5 µm) in enormous numbers that are unconfined. They have distinct conidiophores that form fascicles. The branches are broad and flexuous. They may have conidial pigments that are white or brilliant green. Most Trichoderma strains do not reproduce sexually and instead produce asexual

[Citation: Fatima, A., Khan, Y.S.A., Iqra, Talha, A., Jan, A., Asad, Z., Saba, H., Aslam, A.H., Batool, M., Ashraf, Z.U. (2024). Effectiveness of *Trichoderma* species in the management of sugarcane wilt (*Fusarium sacchari*). *Biol. Clin. Sci. Res. J.*, **2024***: 1316*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1316]

spores. When discovered, the sexual stage is found in Ascomycetes of the genus Hypocrea.

Molecular characterization

After morphology isolates were further confirmed by molecular analysis using primers set ITS 1F/ITS4 at 550 base pair were 4 isolates of *Fusarium sachhari* and 3 isolates of *Trichoderma hamatum*, *Trichoderma harzianum* , *Trichoderma asperllum* identified. Phylogenetic trees were constructed by Neighbor-joining method to conclude the evolutionary relationship of isolated sequence (figure 5,6).

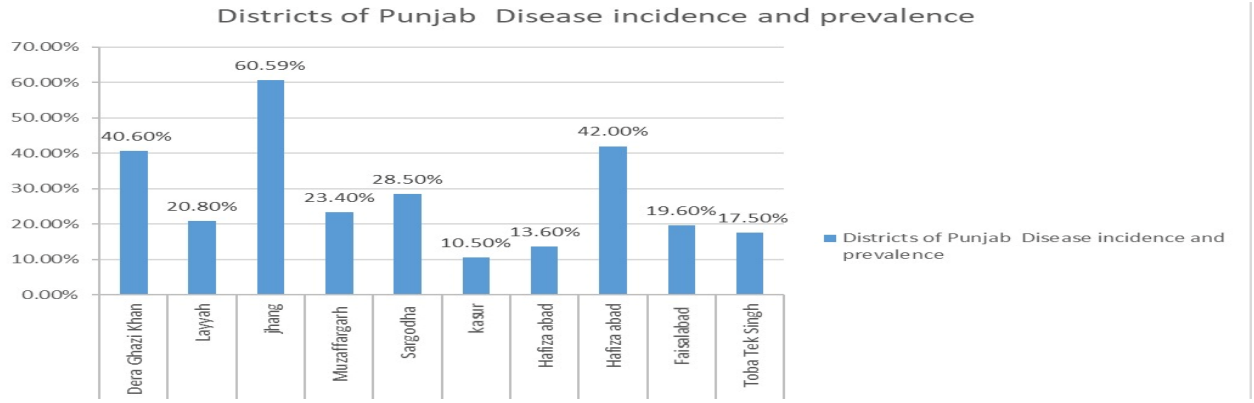


Figure 2 (%) Disease incidence and prevalence of different districts of punjab

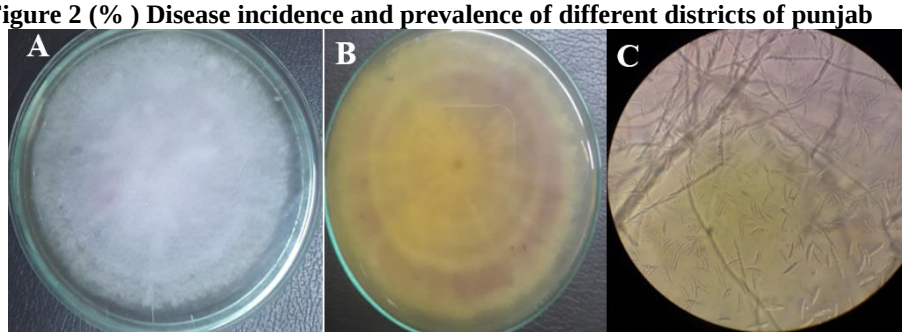


Figure 3. *Fusarium sacchari* culture and spores (A) front view of culture (B) Back view of culture (c) mycelium and spore of *F. sacchari*

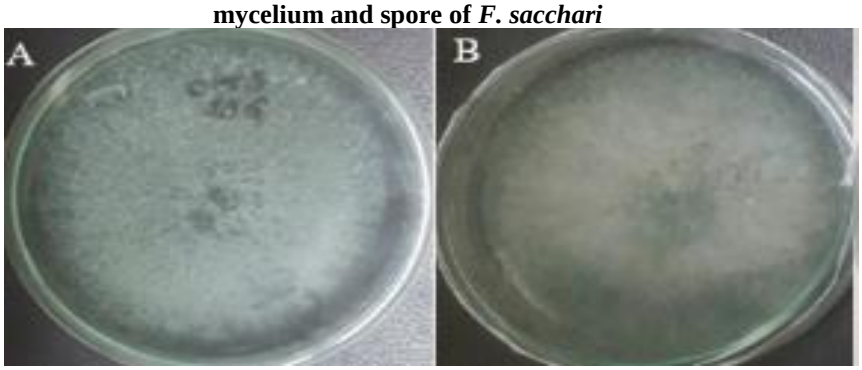


Figure 4: Different Trichoderma spp isolates (A) Trichoderma asperllum (G.M) and (B) Trichoderma hamatum (UN-1)

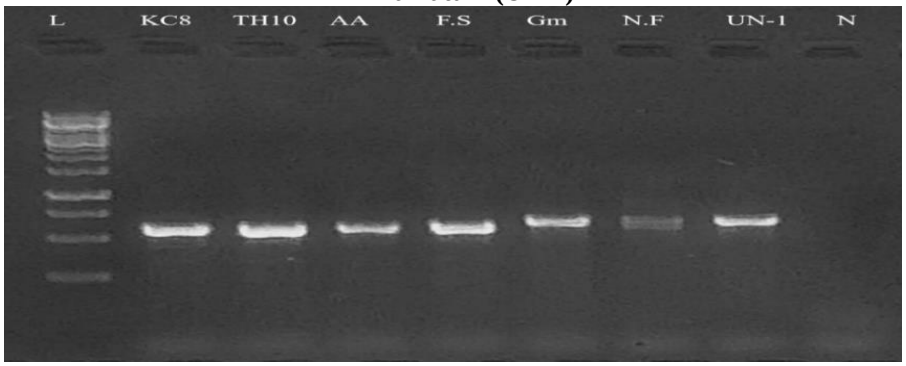


Figure 5: Gel electrophoresis of 4 isolates of *Fusarium sacchari* (F.S, KC-8, AA and TH-10) and *Trichoderma asperrllum* (G.M) and *Trichoderma hamatum* (UN-1*)*

Table 1. List of Fusarium and Trichoderma isolates applied in molecular study

Sample No	Sample name	Sample code	Location
W16	*Fusarium sacchari*	TH10	Sargodha
W17	*Fusarium sacchari*	KC8	Jhang
W18	*Fusarium sacchari*	AA	D.G Khan
W19	*Fusarium sacchari*	FS	Layyah
XO2	*Trichoderma asperllum*	G.M	Garah Mor
X03	*Trichoderma hamatum*	UN-1	Faisalabad

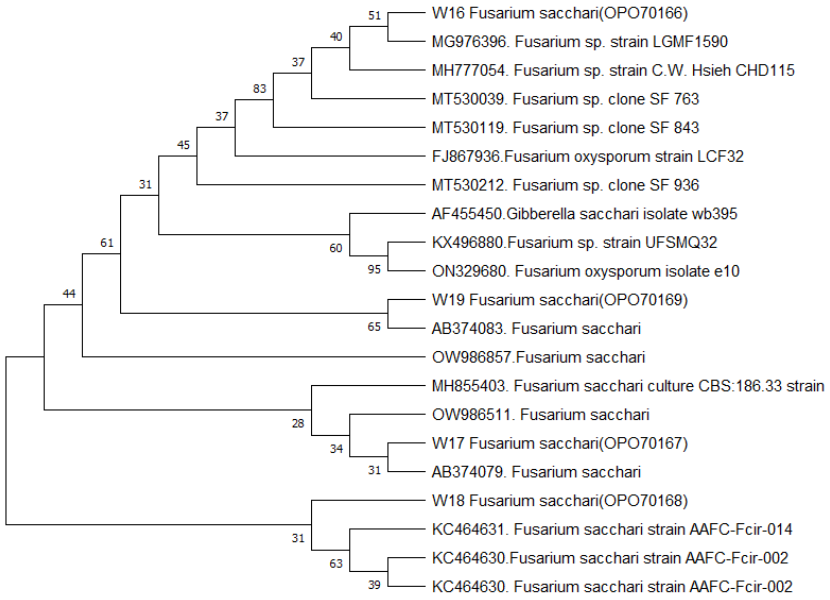


Figure 5. Phylogenetic tree constructed by Neighbor-joining method. To conclude the evolutionary relationship of isolated sequence, 17 related sequences used

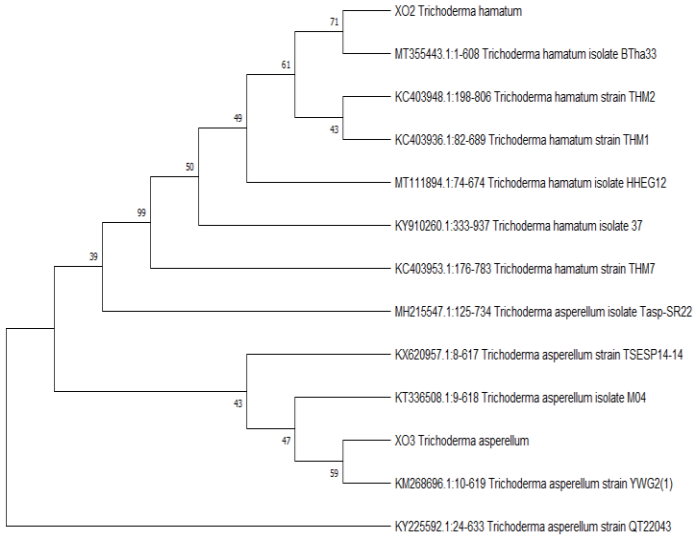


Figure 1. Phylogenetic tree constructed by Neighbor-joining method. To conclude the evolutionary association of isolated sequence of Trichoderma species, 10 related sequences used.

In vitro effect of antagonistic fungi against *Fusarium sacchari*

Dual culture test for inhibition of Fusarium sacharri was conducted (figure 7). The best three isolates of

Trichoderma to inhibit the growth of pathogen is N.F, UN-1, G.M, percentage inhibition for *Trichoderma hamatum* (UN-1), *Trichoderma asperllum* (G.M) and *Trichoderma harzianum (N.F)* is 66%, 75.3%, 71.3% as represented in figure 8.

Interaction between fungal antagonists and days against Fusarium sacchari causing wilt disease is represented in table 2. Results of ANOVA regarding effect of Antagonistic strains against Fusarium sacchari causing sugarcane wilt in dual culture method showed highly significant results as presented in table 3.

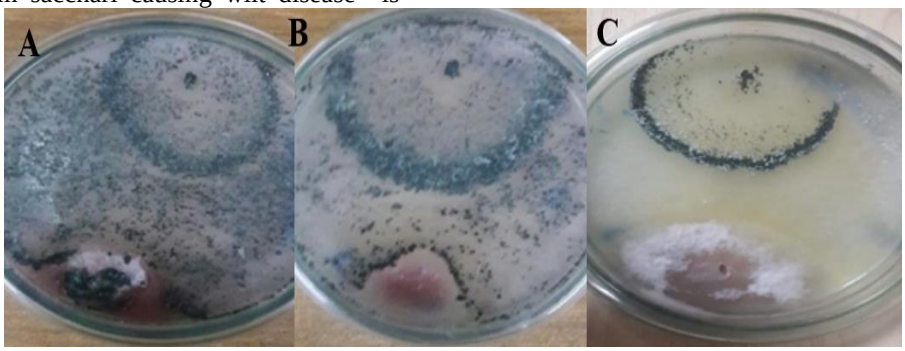


Figure 2. Dual culture test for inhibition of Fusarium sacharri. Through fungal antagonism(A)*Trichoderma asperllum* (G.M) showing 75.33% inhibition.(B) *Trichoderma hamatum* (UN-1) showing 66% inhibition.(C) *Trichoderma harzianum* (N.F) Showing 71.3% inhibition.

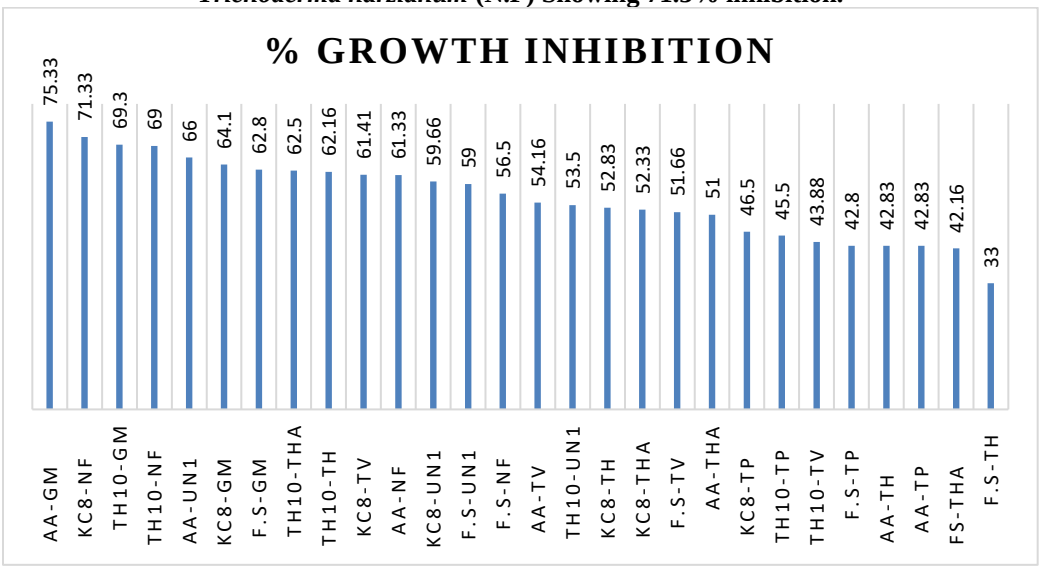


Figure 3. Average percentage of growth inhibition of antagonistic strains against *Fusarium sacchari*

Table 2. Interaction between fungal antagonists and days against *Fusarium sacchari* causing wilt disease

Antagonistic Strain ID	3 days Percentage (%) inhibition	7 Days percentage (%)inhibition
AA-GM	69.667 bcdefghi	81a
AA-NF	51.6 pqrstuv	71 bcdefg
AA-TH	36.66 z	49 rstuvw
AA-THA	46 vwxy	57.3 mnopq
AA-TP	39.33 yz	45.3 vwxy
AA-TV	48 stuvwx	60.33 lmno
AA-UN1	65 efghijklm	67 cdefghijkl
F.S-GM	48.3 stuvwx	77.33 ab
F.S-NF	49.333 qrstuvw	63.66 fghijklmn
F.S-TH	25.6 a	40.333 xyz
F.S-TP	39.3 yz	48 stuvwx
F.S-TV	43.3 wxyz	61.33 jklmno
F.S-UN1	48.6 rstuvw	69.3 bcdefghij

[Citation: Fatima, A., Khan, Y.S.A., Iqra, Talha, A., Jan, A., Asad, Z., Saba, H., Aslam, A.H., Batool, M., Ashraf, Z.U. (2024). Effectiveness of *Trichoderma* species in the management of sugarcane wilt (*Fusarium sacchari*). *Biol. Clin. Sci. Res. J.*, **2024***: 1316*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1316]

FS-THA	42.33 wxyz	42 wxyz
KC8-GM	56.66 nopqr	71.66 bcdef
KC8-NF	69 cdefghijk	73.66 abcd
KC8-TH	49 rstuvw	58 mnop
KC8-THA	47.6 tuvwx	58 mnop
KC8-TP	46.33 uvwxy	55.6 nopqrst
KC8-TV	61.16 klmno	61.66 ijklmno
KC8-UN1	56 nopqrs	63.33 ghijklmn
TH10-GM	63.66 fghijklmn	75abc
TH10-NF	63.3 ghijklmn	72.66 bcde
TH10-TH	54.33 opqrstu	70b cdefgh
TH10-THA	58.33 mnop	66.6 defghijkl
TH10-TP	43.6 vwxyz	49.33 qrstuvw
TH10-TV	42 wxyz	49 rstuvw
TH10-UN1	45.6 vwxy	62.66 hijklmn

*Mean sharing common letters do not differ significantly by HSD test.

Table 2. Analysis of variance regarding effect of Antagonistic strains against *Fusarium sacchari* causing sugarcane wilt in dual culture method

Source	DF	SS	MS	F	P
Replication	2	43.1	21.57		
Strain	27	16882	625.29	24.85	0.0000
Days	1	5164.8	5164.83	205.22	0.0000
Strain × Days	27	1652.5	61.21	2.43	0.0006
Error	110	2768.4	25.17		
Total	167	26511.8			

Grand Mean =55.908 CV =8.97

Pathogenicity test

In this experiment used seven isolates of *Fusarium sacharri* respectively (AA, TH-10, KC8, F.S, S4, M4, S3) with concentration $2x10^6$ and 3 replications (figure 9). After 30 days the best disease incidence were observed in TH-10, KC8, AA, F.S, 71.6%, 60.8%, 68% and 79.3% with $2x10^6$ concentration on the basis of symptoms observed in leaves and re-isolation of pathogen by Koch postulate (table 4, figure 10).In table 5, analysis of variance regarding effect in pathogenicity test showed highly significant results. While in figure 11, average percentage of disease incidence of different *Fusarium sacchari* isolates for pathogenicity test is shown.

Figure 4. Comparison of growth between healthy plants and plants inoculated with Fusarium spp. for pathogenicity test (A) Plants showing wilting symptoms (B) Control plants

Table Error! No text of specified style in document.**. Reading after 15 and 30 days in pathogenicity test different isolates of *Fusarium sacchari***

Treatment	Strain ID	15 days % incidence	30 days % incidence

T1	TH-10	55.16 ef	71.66 ab
T2	KC8	47.06 fg	60.8 6 cde
T3	AA	58.50 de	68.10 bc
T4	FS	64.66 bcd	79.33 a
T5	S4	33.06 hi	37.83 gh
T6	M4	26.76 i	32.43 hi
T7	S3	17.16 j	25.66 ij

*Mean sharing common letters do not differ significantly by HSD test.

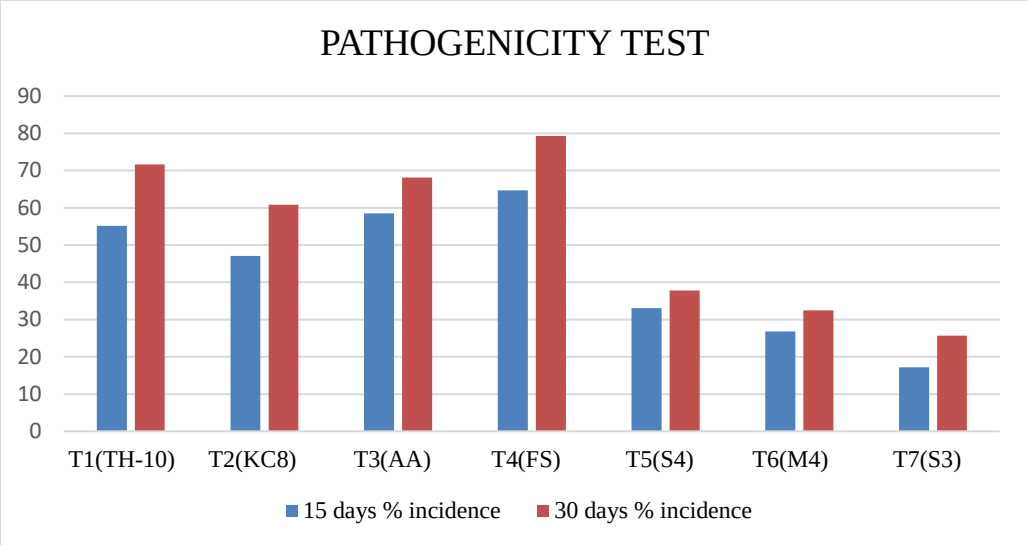


Figure 5. Reading after 15 and 30 days in pathogenicity test.

Table 3. Analysis of variance regarding effect in pathogenicity test

Source	DF	SS	MS	F	P
Replication	2	13.5	6.77		
Treatment	6	13708.1	2284.68	230.59	0.0000
Days	1	1157.6	1157.63	116.84	0.0000
Treatments × Days	6	187.9	31.32	3.16	0.0183
Error	26	257.6	9.91		
Total	41	15324.8			

Grand Mean =48.450 CV =6.50

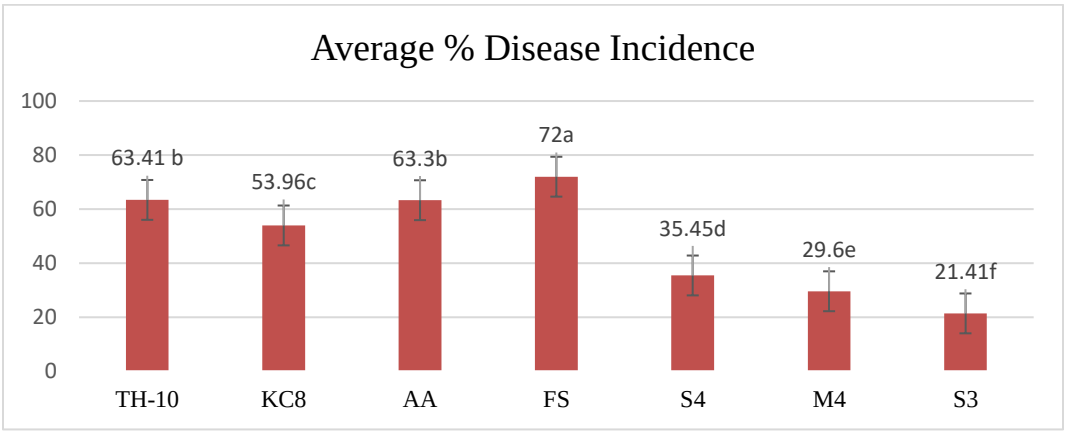


Figure 6. Average percentage of Disease incidence of different *Fusarium sacchari* isolates for pathogenicity test

Efficacy of trichoderma spp. against sugar cane wilt disease (pot experiment)

After 160 days, the best results showed in treatment three consortium of *Trichoderma* species and *Fusarium sacchari* with concentration 2 applied at same time in soil. In this treatment suppressed the wilt disease as compare to disease control plants (figure 12). Disease incidence was recorded in this treatment is 10.6% and disease reduction was recorded 80%. In second treatment with concentration two, disease incidence was recorded 45.3% and disease reduction was recorded 59.3%. In treatment first with concentration two, disease incidence was recorded 45% and disease reduction was recorded 35.6%. In disease control treatment four, disease incidence was recorded 71%. Average disease incidence was recorded in both concentration is 48% (c^1) and 71% (c^2) (figure 13, 14 and 15). Diseased plants showed wilt symptoms. In treatment five consortium of *Trichoderma* species control plants showed better efficacy as compared to disease control. Plants height is greater than diseased plants and look healthy and fresh.

Figure 7. Efficacy of *Trichoderma* species A) Pot experiment check efficacy of *Trichoderma asperllum (G.M), Trichoderma hamatum (UN-1) and Trichoderma harzianum* (N.F). against wilt, disease (B) Disease control (C) Comparison of disease control and healthy control (D) Comparison of healthy control and Trichoderma species control (E) Trichoderma species plants showed their efficacy

The result of pot experiment with both concentration show their result with disease incidence and disease reduction after 160 days (figure 16). Results of analysis of variance regarding effect of Antagonistic strains against Fusarium sacchari in pot experiment (Disease Reduction) showed highly significant results.

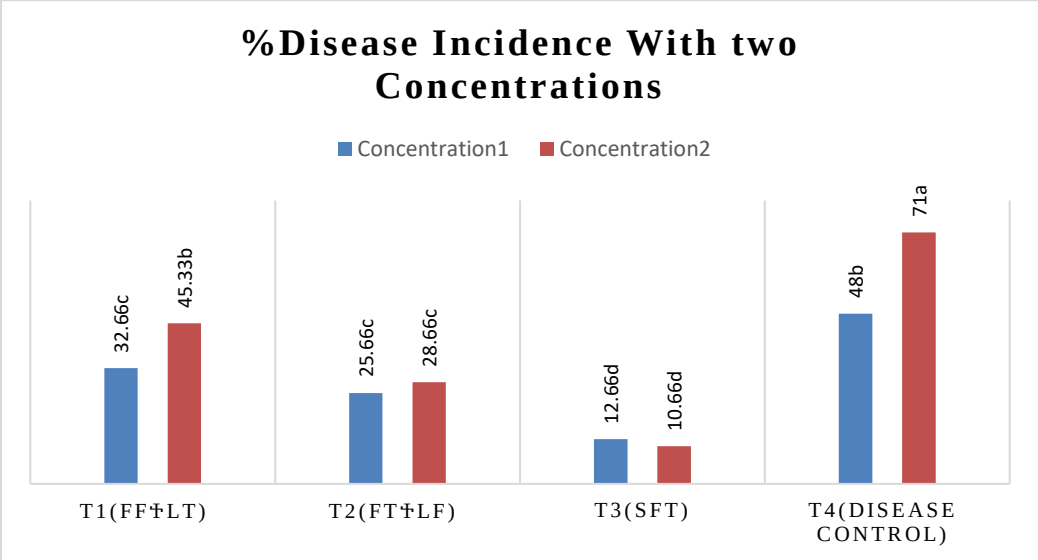


Figure 8. Efficacy of *Trichoderma* species against wilt disease with both average concentrations showing disease incidence

Table 4. Analysis of variance regarding effect of Antagonistic strains against *Fusarium sacchari* in pot experiment (disease incidence)

[Citation: Fatima, A., Khan, Y.S.A., Iqra, Talha, A., Jan, A., Asad, Z., Saba, H., Aslam, A.H., Batool, M., Ashraf, Z.U. (2024). Effectiveness of *Trichoderma* species in the management of sugarcane wilt (*Fusarium sacchari*). *Biol. Clin. Sci. Res. J.*, **2024***: 1316*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1316]

Source	DF	SS	MS	F	P
Concentrations	2	104.08	52.04		
Treatment	3	7321.67	2440.56	407.16	0.0000
Days	1	504.17	505.17	84.11	0.0000
Treatment × Days	3	549.50	183.17	30.56	0.0000
Error	14	83.92	5.99		
Total	23	8563.33			

Grand Mean = 34.333 CV= 7.13

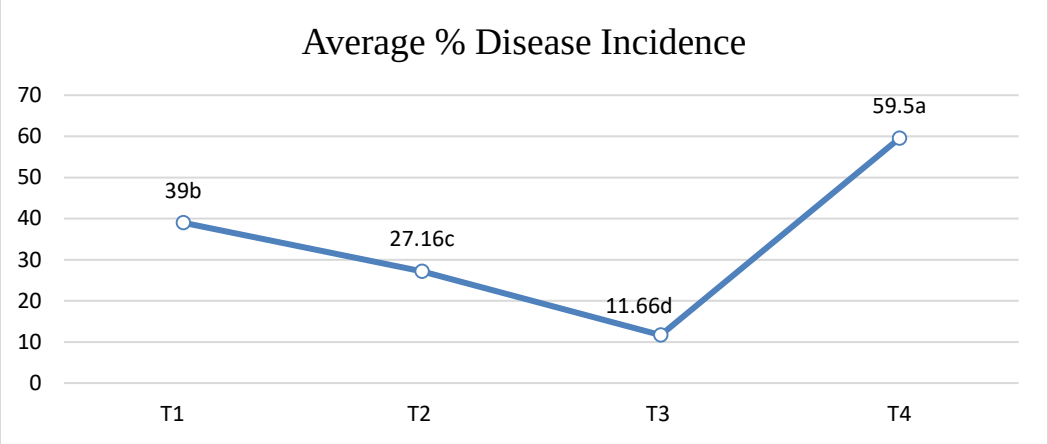


Figure 9: Average percentage disease incidence to check the antagonistic effect in different treatments.

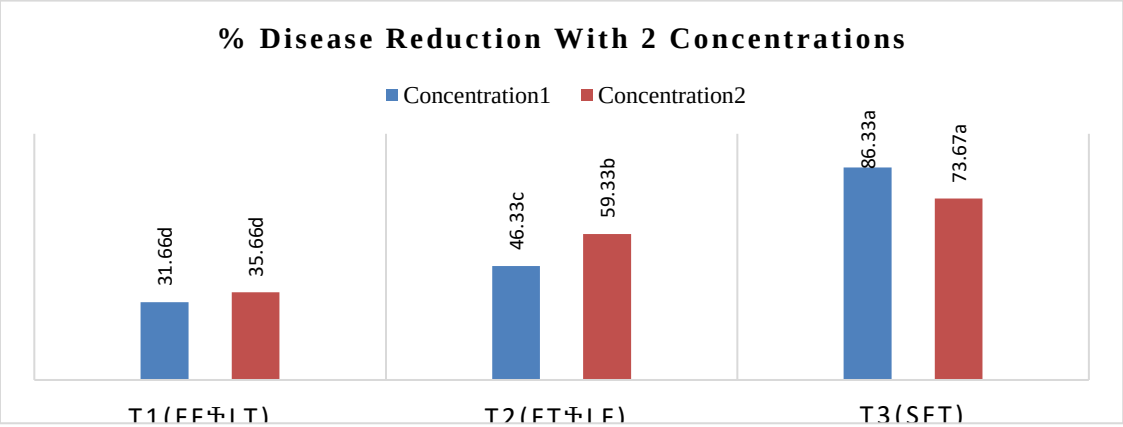


Figure 10. Efficacy of *Trichoderma* species against wilt disease with both average concentrations showing disease reduction

Table 5. Analysis of variance regarding effect of Antagonistic strains against *Fusarium sacchari* in pot experiment (Disease Reduction)

Source	DF	SS	MS	F	P
Concentrations	2	26.33	13.17		
Treatment	2	6504.33	3252.17	153.65	0.0000
Days	1	440.06	440.06	20.79	0.0000
Treatment × Days	2	78.11	39.06	1.85	0.2079
Error	10	211.67	21.17		
Total	17	7260.50			

Grand Mean = 55.50 CV= 8.29

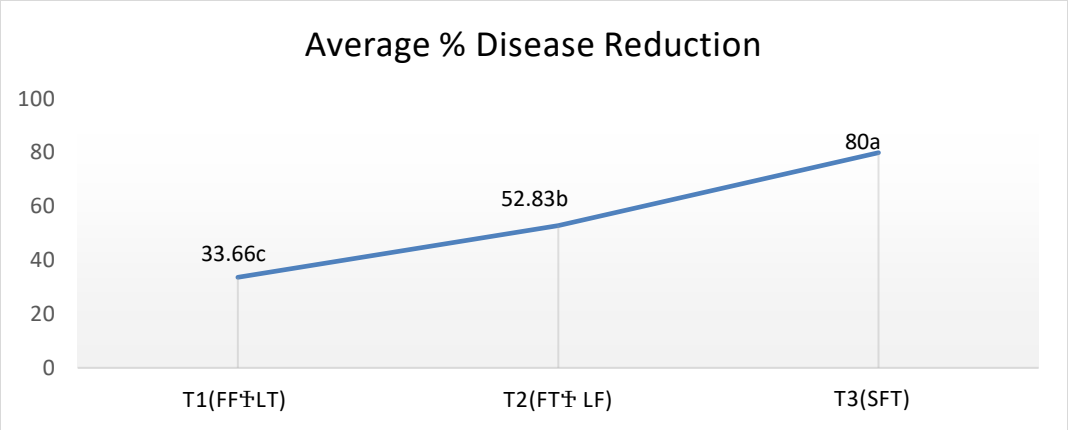


Figure 11. Average percentage disease reduction to check the antagonistic effect in different treatments

Discussion

Various biotic and abiotic elements in the field influence the manifestation and severity of diseases. In the previous study, scientists discovered wilting symptoms in Pakistan's Punjab, Sindh, and KPK provinces (Nisa. S.G et al., 2022). The morphological study and identification of fungi is important for identifying isolates up to the family or genera level. Fungi species identification is critical in both basic (ecology, taxonomy) and functional (genomics, bioprospecting) scientific research applications. In the present study, the morphological characteristics and microscopic study antagonistic and fungal cultures identified through identification key. In the previous study scientist also worked on the identification of *F. Leslie and Summerell (2006) did sacchari based on the taxonomic guidelines*. Molecular identification is important to find precise species of fungi. Molecular analysis using primers set ITS-1F/ITS4 at 550 base pair were 4 isolates of *Fusarium sachhari* and 3 isolates of *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma asperllum* identified. In a previous work, O'Donnell et al. (1998) used a TEF1-α gene-specific primer to identify a Fusarium isolate, yielding an amplicon of 656 base pairs. Pathogenicity test was done. After 30 days the best disease incidence were observed in TH-10, KC8, AA, F.S is 71.6%, 60.8%, 68% and 79.3% with $2x10^6$ concentration on the basis of symptoms showing in leafs and re-isolation of pathogen by Koch postulate.

Trichoderma species are already reported biological agents that have previously been used to treat Fusarium wilt in a number of crops of crops. Trichoderma spp. are soil-inhabiting fungus that have been employed to manage plant diseases. In our study, the *Trichoderma harzianum* showed highest percentage inhibition of 81% followed by *Trichoderma asperllum* 73% and *Trichoderma harzianum (un-1)* 63.3% against pathogen. In a study conducted by Sundarmoorthy and Balabaskar (2013), the effectiveness of *Trichoderma* against *F. oxysporum*, the fungus responsible for the wilt disease that affects tomato, was evaluated as was reported effective. In a study that reached a similar conclusion, Adhikary et al. (2017) reported that *Trichoderma* treatment significantly reduced the disease symptoms in eggplant (Solanum melongena L.). Trichoderma isolates were tested in vitro against the fungus *Fusarium oxysporum*, which is the agent responsible for chickpea wilt disease. Anuragi and Sharma (2016) found that T. reesei reported antagonistic activity for *F. oxysporum.* Dual cultures of *Fusarium* were followed by *T. viride* cultivation. in addition to *T. harzianum*. However, despite the fact that a number of studies have been arranged to evaluate the potential of *Trichoderma* in other crops, there have only been a very small number of studies conducted to investigate the effectiveness of *Trichoderma* in combating sugarcane wilt. In a few of these earlier studies, the primary emphasis was placed on determining the antagonistic potential of six to seven isolates of *Trichoderma* against *Fusarium* in vitro, and it was found that potent isolates existed (Gawade et al., 2012). Sugarcane wilt is believed to be caused by *Fussarium sacchari*, which is now known to be the causative agent. When it comes to managing sugarcane wilt, the use of potent endophytic *Trichoderma* strains, as opposed to rhizospheric *Trichoderma* strains, can give an added advantage. This is due to the fact that sugarcane wilt is primarily a soil-borne disease. The endophytic genotypes will be better adapted to populate themselves and inhibit the target pathogen within the plant tissue, which may function better in soil and the rhizosphere of the plant. This is because endophytic strains are more closely associated with the plant. A number of endophytic *Trichoderma* strains have been utilized in the fight against wilt caused by *Fusarium* in crops other than sugarcane. For example, Dolatabadi et al. (2012) reported that root endophytic strains of *Trichoderma* were able to suppress *F. sacchari*, the fungus responsible for the wilt disease that affects lentils. In a similar manner,

[Citation: Fatima, A., Khan, Y.S.A., Iqra, Talha, A., Jan, A., Asad, Z., Saba, H., Aslam, A.H., Batool, M., Ashraf, Z.U. (2024). Effectiveness of *Trichoderma* species in the management of sugarcane wilt (*Fusarium sacchari*). *Biol. Clin. Sci. Res. J.*, **2024***: 1316*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1316]

Taribuka et al. (2017) tested endophytic *Trichoderma* isolates against *Fusarium sacharri*, the fungus that causes wilt disease in bananas.

Trichoderma play important role in antibiosis, mycoparasitism, promotion of plant growth, induced plant defense mechanisms, and amendment of environment. To check the efficacy of *trichoderma* against wilt disease in pots. In treatment three consortium of *Trichoderma* strains and *fusarium sacharri* with concentration 2 applied at same time showed better efficacy as compared to other treatments. Disease reduction was recorded 85% and disease incidence was recorded 10 % in this treatment after 160 days. *Trichoderma* and *fusarium spp* both are present in soil so when *fusrium* spp attack on crops *Trichoderma spp.* enter the pathogen-produce enzymes to controlling the soil borne pathogen so, this treatment showed better efficacy as compared to other treatments to control wilt disease. In disease control, plant disease incidence was recorded 85%. Similar results were recorded by According to Gawade et al. (2012), the study found that various Trichoderma isolates have a strong antagonistic effect on Fusarium sacharri mycelium growth. Sabalpara et al. (2009) reported that Trichoderma were examined for the goal of reducing sugarcane wilt and root rot pathogens and obtaining effective isolates. The isolates were cultured on Trichoderma special medium, and a pressmud-based formulation was created for large-scale field application. In addition, it was shown that various strains of the same species were inhibited to varying degrees. A variety of isolates of *Trichoderma harzianum* parasitized *S. rolfsii*, as reported by Henis and colleagues 1983. The TCVSI-1 (18.00 mm) and TCVSI-3 (6.00 mm) were suppressed by all three isolates of the pathogen in concentrations of 25, 50, and 75 percent, but *T. harzianum* and 100 percent concentration were shown to be the most potent at 100 percent concentration by Dharmaputra and his study team 1994. As a whole, the inhibition performed the best. LCF increased *Macrophomina* concentration in all treatments, according to Etabarian, 2006 although *T.viridae* decreased colony area. TCVSI-1 LCF performed best among six phaseoli by 19.2% and 34.9% using dual culture and *Trichoderma* species cellophane techniques. All three concentrations of Ghisalberti, PIMG, and TCVSI-3. Other than mycelium, Rowland, 1993, found nothing in a concentration greater than one hundred percent. The *Trichoderma's* metabolites resulting from interactions and hyperparasitism. The activity of produced by basidiomycetes in freshly fallen pine antibiotic metabolites in *Trichoderma* could impact the outcome of decay species, and scientists have recognized this as a contributing factor in the. Biocontrol of plant diseases by Dennis and Webster. Dennis and microorganism have described the potential of *Trichoderma* to produce inhibitory chemicals in culture filtrate against *Trichoderma* and inhibitory substances against microorganisms. Webster is a producer of antibiotics.

References

Abbas, A., Rashad, A., Rehman, A., & Bukhari, M. (2024a). Exploring the response mechanisms of rice to salinity stress. *Bulletin of Biological and Allied Sciences Research*, **2024**(1), 58. https://doi.org/10.54112/bbasr.v2024i1.58

Abbas, A., Arshad, A., Rehman, A., Bukhari, M., & Zaman, S. (2024b). Revolutionizing plant breeding programs with advancements in molecular marker-assisted selection. *Bulletin of Biological and Allied Sciences Research*, **2024**(1), 57. https://doi.org/10.54112/bbasr.v2024i1.57

Adhikary, M. C., Begum, H. A., & Meah, M. B. (2017). Possibility of recovering Fusarium Wilt affected eggplants by Trichoderma. *International Journal of Agricultural Research, Innovation and Technology*, ***7***(1), 38-42.

Ahmad, B., Yunxian, Y., Ur Rahman, Z., Gultaj, H., Naseem Siddiqui, B., Ali, M., & Nasar, J. (2022). Enhancement of sugarcane production by counteracting the adverse effects of climate change in Sindh Province, Pakistan. *Growth and Change*, ***53***(1), 76-90.

Anuragi, M. E., & Sharma, T. K. (2016). Biocontrol of chickpea wilt disease by Fusarium oxysporum f. sp. ciceri with rhizopshere mycoflora. *Flora and fauna*, ***22***(2), 201-209.

Bailey, R. (2004). Diseases. *Sugarcane*, 54-77.

Bhunjun, C.S., Niskanen, T., Suwannarach, N. *et al*. The numbers of fungi: are the most speciose genera truly diverse *Fungal Diversity* **114,** 387–462 (2022). https://doi.org/10.1007/s13225-022-00501-4

Charati, S. N., Mali J. B., and Pawar, N. B. (1998). *J. Maharashtra Agric. Univ*., **23**(3), 194-200.

DEACON, J.W. (2006). Fungal Biology (4th ed.). United Kingdom: Blackwell Publishing Ltd.

Dharmaputra, O. S., Purba, R. Y., & Sipayung, A. (1994, December). Research activities on the biology and control of Ganoderma at SEAMEO BIOTROP and IOPRI Marihat. In *Proceedings of First International Workshop on Perennial Crop Diseases Caused by Ganoderma. Ed. M. Holderness. Universiti Pertanian Malaysia, Selangor*.

Diao YZ, Zhang C, Liu F, Wang WZ, Liu L, Cai L, Liu XL (2017) Colletotrichum species causing anthracnose disease of chili in China. *Persoonia: Molecular Phylogeny and Evolution of Fungi* **38**, 20.

Dolatabadi HK, Goltapeh EM, Mohammadi N, Rabiey M, Rohani N, Varma A (2012) Biocontrol potential of root endophytic fungi and Trichoderma species against Fusarium wilt of lentils under in vitro and green house conditions. *J Agr Sci Tech* **14**, 407-420

Etebarian, H. R. (2006). Evaluation of Trichoderma isolates for biological control of charcoal stem rot in melon caused by Macrophomina phaseolina.

FAO. FAOSTAT, Crops: Sugarcane; 2022 [cited 2022 March 15]. Database: FAOSTAT http://www.fao.org/faostat/en/#data/QC.

Gawade, D. B., Pawar, B. H., Gawande, S. J., & Vasekar, V. C. (2012). Antagonistic effect of Trichoderma against Fusarium moniliformae the causal of sugarcane wilt. *American-Eurasian J Agric & Environ Sci*, ***12***(9), 1236-1241.

Ghisalberti, E.L. and C.Y. Rowland, 1993. Antifungal metabolites from Trichoderma harzianum. *J. Nat. Prod*., **56**: 1799-1804.

Henis, Y., Adams, P. B., Lewis, J. A., & Papavizas, G. C. (1983). Penetration of sclerotia of Sclerotium rolfsii by Trichoderma spp. *Phytopathology*, ***73***(7), 1043-1046.

Irfan, M., Fatima, N., Ali, F., & Haider, M. (2024). Assessing potato cultivation techniques in pakistan: an analysis of existing methods and identified gaps. *Bulletin of Biological and Allied Sciences Research*, **2024**(1), 80. https://doi.org/10.54112/bbasr.v2024i1.80

Jahanshir, A., & Dzhalilov, F. S. (2010) The Effects of Fungicides on Fusarium oxysporum f. sp. Lycopersici associated with Fusarium wilt of tomato. *J Plant Prot Res* **50**(20), 172-178

Karamchandani, B. M., Chakraborty, S., Dalvi, S. G., & Satpute, S. K. (2022). Chitosan and its derivatives: Promising biomaterial in averting fungal diseases of sugarcane and other crops. *Journal of Basic Microbiology*, ***62***(5), 533-554.

Khan, M. E. (2022). Products and by-products of sugarcane in pakistan. *Pakistan Sugar Journal*, ***37***(1), 19.

Liu F, Hou L, Raza M, Cai L (2017). Pestalotiopsis and allied genera from Camellia, with description of 11 new species from China. *Scientific Reports* **7**(1):1–19.

Lockhart, B., Cronjé, C., Rott, P., Bailey, R., Comstock, J., Croft, B., & Saumtally, S. (2000). A guide to sugarcane diseases. *Editions Quae, CIRAD*, 266-272.

Mehnaz, S. (2013). Microbes–friends and foes of sugarcane. *Journal of Basic Microbiology*, **53**(12), 954–971. https://doi.org/10.1002/jobm.201200299

Nisa, S. G., Farooq, M. A., Iqbal, U., Fatima, A., Ahmad, Z., Tabassam, S., Waseem, S., Tassadaq, S., Haroon, U., Chaudhary, H. J., & Munis, M. F. H. (2022). Incidence and detection of wilting pathogen and Sugarcane mosaic virus (SCMV) in Pakistan. *Journal of Phytopathology*, **1**, 1–9. https://doi.org/10.1111/jph.13097.

Okungbowa, F. I., & Shittu, H. O. (2012). Fusarium wilts: An overview. *Environment Research Journal*, **6**(2), 83–102.

Pandey, K. K., & Upadhyay, J. P. (1999). Antagonism of Trichoderma against sugarcane wilt pathogen (Fusarium moniliformae). *J.Mycol. Pl. Pathol*, 29(2): 214-216.

Poongothai, M., Viswanathan, R., Malathi, P., & Sundar, A. R. (2014). Sugarcane wilt: Pathogen recovery from different tissues and variation in cultural characters. *Sugar Tech*, ***16***(1), 50-66.

Prasad, R. D. & Rangeshwaran, R. (2000). *J.Mycol. Pl. Pathol*, **30**(2), 216-220.

Raza M, Zhang ZF, Hyde KD, Diao YZ, Cai L (2019) Culturable plant pathogenic fungi associated with sugarcane in southern China. *Fungal Diversity* **99**(1), 1–104.

Rehman, A., Abbas, A., Arshad, A., Raza, G., Umar, M., & Bukhari, M. (2024). advancements in genomic technologies and their impact on crop improvement and breeding methods. *Bulletin of Biological and Allied Sciences Research*, *2024*(1), 61. https://doi.org/10.54112/bbasr.v2024i1.61

Rini, C., & Sulochana, K. (2008). Usefulness of Trichoderma and Pseudomonas against Rhizoctonia solani and Fusarium oxysporum infecting tomato. *Journal of Tropical Agriculture*, ***45***(1), 21-28

Sabalpara, A. N., Priya, J., Waghunde, R. R., & Pandya, J. P. (2009). Antagonism of Trichoderma against sugarcane wilt pathogen (Fusarium moniliformae). *Am-Eurasian J Sustain Agric*, ***3***(4), 637-638.

Sami, A., Haider, M., Meeran, M., Ali, M., Abbas, A., Ali, Q., & Umar, M. (2023). exploring morphological traits variation in chenopodium murale: a comprehensive multivariate analysis. *Bulletin of Biological and Allied Sciences Research*, **2023**(1), 43. https://doi.org/10.54112/bbasr.v2023i1.43

Sharma, P., Sharma, M., Raja, M., & Shanmugam, V. (2014). Status of Trichoderma research in India: *A review. Indian Phytopathol*, **67**(1), 1-19.

Singh RS. Plant Diseases. 9th Edition. Oxford and IBH Publishing; 2018

Sundaramoorthy, S., & Balabaskar, P. (2013). Biocontrol efficacy of Trichoderma spp. against wilt of tomato caused by Fusarium oxysporum

f. sp. lycopersici. *Journal of Applied Biology and Biotechnology*, ***1***(3), 0-4.

Taribuka J, Wibowo A, Widyastuti S, Sumardiyon C (2017) Potency of six isolates of biocontrol agents endophytic Trichoderma against Fusarium wilt on banana. *J Degrade Min Land Manage* **4**(2), 723-731

Teng, P. S. (1983). Estimating and interpreting disease intensity and loss in commercial fields. *Phytopathology*, **73**(11), 1587–1590. https://doi.org/10.1094/Phyto-73-1587

Tiwari, R., Chandra, K., Shukla, S.K. *et al.* Interference of bio-control *Trichoderma* to enhance physical and physiological strength of sugarcane during Pokkah boeng infection. *World J Microbiol Biotechnol* **38,** 139 (2022).

Viswanathan, R. & Malathi, P. (2018). Biocontrol Strategies to Manage Fungal Diseases in Sugarcane. *Sugar Tech* https://doi.org/10.1007/s12355-018-0690-3

Viswanathan, R. (2013). Status of sugarcane wilt: One 100 years after its occurrence in India. *Journal of Sugarcane Research* **3** (2), 86–106.

Viswanathan, R., & Rao, G. P. (2011). Disease scenario and management of major sugarcane diseases in India. *Sugar Tech* **13** (4): 336–353.

Viswanathan, R., Poongothai, M., & Malathi, P. (2011). Pathogenic and molecular confirmation of Fusarium sacchari causing wilt in sugarcane. *Sugar Tech*, **13**(1), 68–76.

Viswanathan, R., Poongothai, M., Malathi, P., & Prasanth C. N. (2015). Sugarcane wilt: simulation of pathogenicity through diferent methods and environments. *Intern Sugar J* **117**, 286–293

Declaration

Acknowledgement
Not applicable

Ethics Approval and Consent to Participate
Not applicable.

Consent for Publication
The study was approved by authors.

Funding Statement
Not applicable

Authors' Contribution
All authors contributed equally.

Conflict of interest
There is no conflict of interest among the authors of the manuscript.