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IN VITRO ANTAGONISTIC EFFECTS OF *TRICHODERMA HARZIANUM* AGAINST WHEAT-BORNE FUNGAL PATHOGENS

[a]Ayesha Bibi*, Najam U.S. Afshan[a], Fouzia Yaquob[b]

[a] *Institute of Botany, University of Punjab, Lahore, Pakistan.*
[b] *Department of Botany, University of Sargodha, Sargodha, Pakistan.*

A B S T R A C T

Biological control of fungi is a potential alternative to the use of chemical pesticides, which have been proved to be harmful to the environment. Pathogenic fungi were isolated and identified from wheat on PDA medium. *Trichoderma harzianum* have been isolated from soil and found to be effective biocontrol agent against various wheat-borne pathogenic fungi. *Trichoderma harzianum* inhibited the growth of wheat-borne fungi like *Aspergillus, Alternaria triticina, Fusraium solani, Rhizopus oryzae, Rhizactonia solani, Sclerotium rolfsii* and *Bipolaris* in dual culture plate assay.

Keywords: Biological control, Wheat-borne pathogens and *Trichoderma harzianum*.

INTRODUCTION

Wheat (*Triticum aestivum* L.) belongs to family *Gramineae* and it is considered as major food crop. It ranks first as necessary food crop in Pakistan, after that rice and maize are important. Pakistan has to trade in wheat considerably from different countries to fulfil the needs of people (Hussain *et al*., 2012). During the year 2020, wheat production for Pakistan was 25,700 thousand tonnes with an average annual rate of 3.18% was recorded.

Fungi that effect wheat which are seed borne comprise *Alternaria alternata, Cladosporium oxysporum*, *Curvularia lunata*, *Fusarium solani,* and fungi that are post harvested comprise *Aspergillus niger* (Ilyas *et al.*, 1998). *Bipolaris*, *Alternaria*, *Rhizopus*, *Aspergillus, Fusarium*, and *Rhizactonia* are the most common fungi. These pathogenic fungi were isolated from seeds of wheat (Rehman *et al*., 2011).

Soil borne fungal pathogens infect number of plants. The most important genera include *Alternaria*, *Armillaria*, *Aspergillus*, *Chaetomium*, *Cylindrocladium*, *Fusarium*, *Geotrichum*, *Penicillium*, *Phytophthora*, *Pythium*, *Rhizoctonia* and *Sclerotinia* (Azaz, 2003).

Species of *Fusarium, Pythium*, *Phytophthora*, *Rhizoctonia*, and *Sclerotium* fungi affect the seeds during germination, pre and post emergence phases of seedling establishment (Lichtenzveig *et al*, 2006).

The antagonistic potentiality of *Trichoderma* species as bio control agents for plant diseases was first recognized in the early 1930s (Howell, 2003). *Trichoderma harzianum* is very effective in controlling plant pathogens. It was commercially produced to stop the growth of several soil born pathogenic fungi (Khan *et al*., 2004; Mukhtar *et al*., 2013, 2018. 2021; Iqbal *et al*., 2020; Azeem *et al*., 2020).

Trichoderma spp. has been very efficient in decreasing foliar disease severity in wheat plants as compare to plants that are not treated (Sabatini *et al*., 2002; Perello *et al*., 2003; Muthomi *et al*., 2007). The objective of the present investigation was isolation and screening of *Trichoderma harzianum* against the fungal pathogens.

MATERIAL AND METHODS

Isolation and identification of wheat-borne fungal pathogens*: Fusarium solani, Alternaria triticina,*

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* Corresponding Author:
Email: aisha.study.abroad@gmail.com

Aspergillus niger, Sclerotium rolfsii, Bipolaris, Rhyzopus oryzae, and *Rhizactonia solani* fungi were isolated and identified according to method of Harrigan (1998) from wheat at different stages of growth on PDA.

Isolates used in Study: Cultures of *Trichoderma harzianum* used as biological control agent were isolated from soil and identified according to method of Harrigan (1998).

***In vitro* Antagonistic activity of *T. harzianum* against wheat-borne fungal pathogens:** Cultures of *Trichoderma harzianum* used as antagonist were inoculated on Petri plates. A disc was taken with the help of corkborer from 7 day old cultures of *Fusarium solani, Alernaria triticina, Aspergillus niger, Sclerotium rolfsii, Bipolaris, Rhyzopus oryzae,* and *Rhizactonia solani* was placed on a fresh PDA plate and incubated at 28°C for 72 to 96 hrs to allow pathogen to grow.

A disc of *Trichoderma harzianum* was placed at one side of Petri plates and a disc of fungal isolates cut from previous plates was placed at the opposite side. These plates were incubated at room temperature and colony growth from the edge of inoculum disc to the colony margin recorded daily until both the microorganisms come in contact with each other or a zone of inhibition was formed, which was measured.

Percentage inhibition of mycelia growth of Wheat-borne fungal pathogens by *Trichoderma harzianum* was calculated with respect to growth of the control by using following formula:

$$\text{\% Inhibition of radial mycelial growth} = \frac{C - T}{C} \times 100$$

Where, C = measurement of the wheat borne fungal pathogen in control

T = growth of the pathogen in the presence of *Trichoderma harzianum* in dual culture

RESULTS

Isolation and identification of wheat born fungal pathogens: Wheat born fungal pathogens were isolated from soil, roots, stem and leaves of the infected plants. After purification, these isolates were identified on the basis of colony characters, morphology and growth pattern. The identified isolates from soil, leaves, stem and roots were shown in Table 1. The identified isolates from roots were *Fusarium solani*, *Bipolaris*, *Rhizactonia solani,* and *Aspergillus niger*. The isolates from stem and leaves of the plant were identified as *Sclerotium rolfsii* and *Alternaria triticina*. While other fungal pathogens that are isolated from soil of wheat field were *Rhizopus oryzae and Phytophthora infestans.* Both of these pathogens are not wheat borne. Antagonistic effect of *Trichoderma harzianum* showed significant growth inhibition of all fungal pathogens in dual culture.

Types of *In vitro* interaction of *Trichoderma harzianum* with fungal pathogens in dual nature are shown in Table 2. *Trichoderma harzianum* found to be effective against inhibiting the growth of almost all fungal pathogens in dual culture plate assay and zone of inhibition is formed.

All the fungal isolates in PDA media are shown in Fig 1. *Trichoderma harzianum* show significant growth inhibition of all pathogens in dual culture is shown in Figure 2. Inhibition percentage of *Trichoderma harzianum* against isolated fungal pathogens is given in Fig 3. Percent inhibition of fungal pathogens shows the effectiveness of *Trichoderma harzianum.* The overall results revealed that the maximum inhibition was observed in case of *Phytophthora infestans* with mean inhibition of 64.92% followed by the inhibition of *Fusarium solani* with mean value of 62.38%. However, the lowest inhibition was observed in case of *Rhizopus oyzae* with mean inhibition of 19.82%.

Table 1. Fungus isolated from soil, leaves, stem and roots

Fungal Isolates (Soil)	Fungal Isolates (Leaves & Stem)	Fungal Isolate (Roots)
Fusarium solani	*Alternaria triticina*	*Rhizactonia solani*
Rhizopus oryzae	*Sclerotium rolfsii*	*Fusarium solani*
Rhizopus oryzae	*Alternaria triticina*	*Rhizactonia solani*
Fusarium solani	*Sclerotium rolfsii*	*Bipolaris*
Rhizopus oryzae	*Rhizactonia solani*	*Rhizactonia solani*
Aspergillus niger	*Alternaria triticina*	-
Aspergillus niger	*Sclerotium rolfsii*	-
Rhizactonia solani	-	-
Bipolaris	-	-
Aspergillus niger	-	-
Phytophthora infestans	-	-

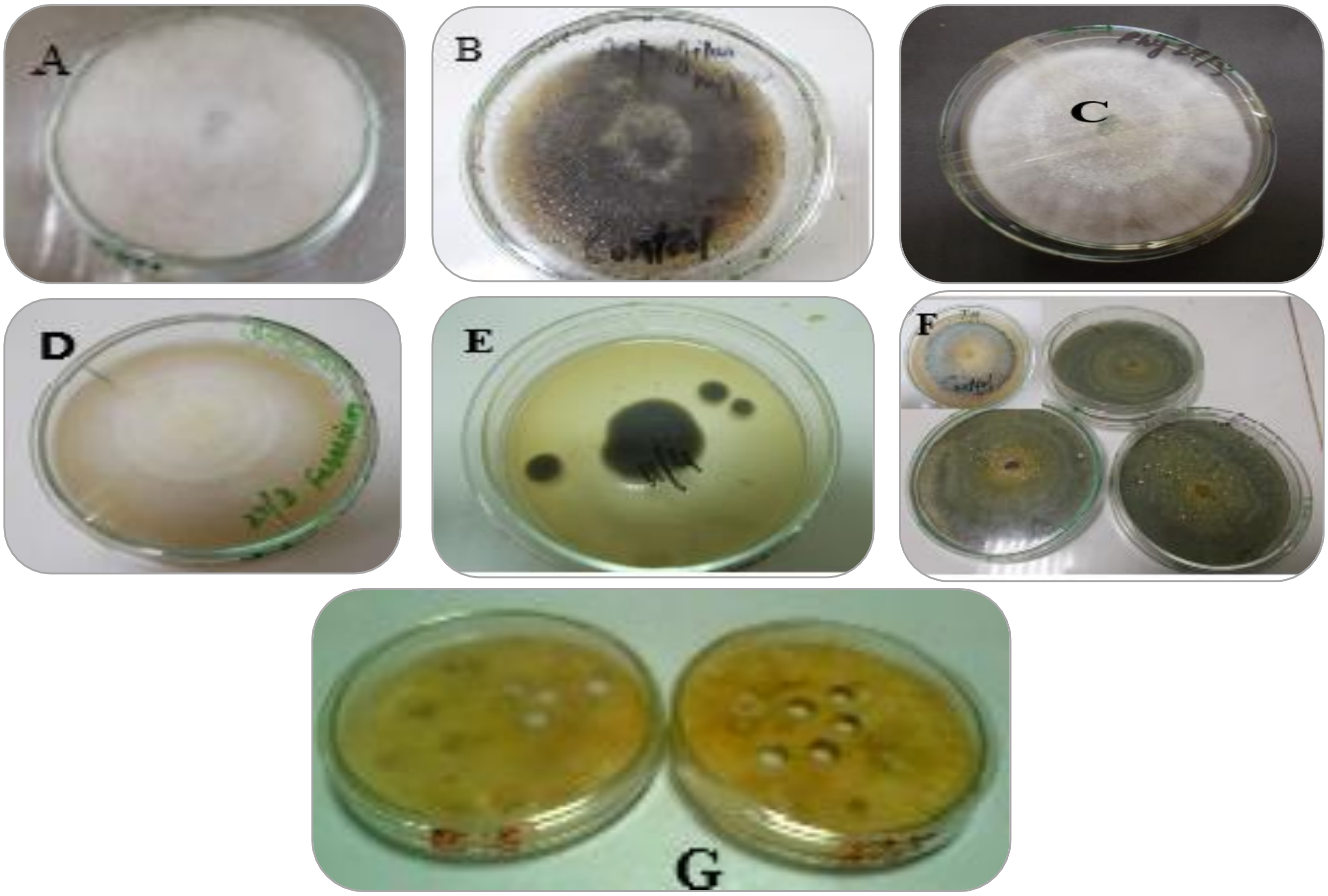

Figure 1. A=*Rhizopus oryzae* B=*Aspergillus niger* C= *Phytophthora infestans* D= *Fusarium solani* E= *Alnernaria spp.* F= *Trichoderma harzianum* G= *Rhizactonia solani*

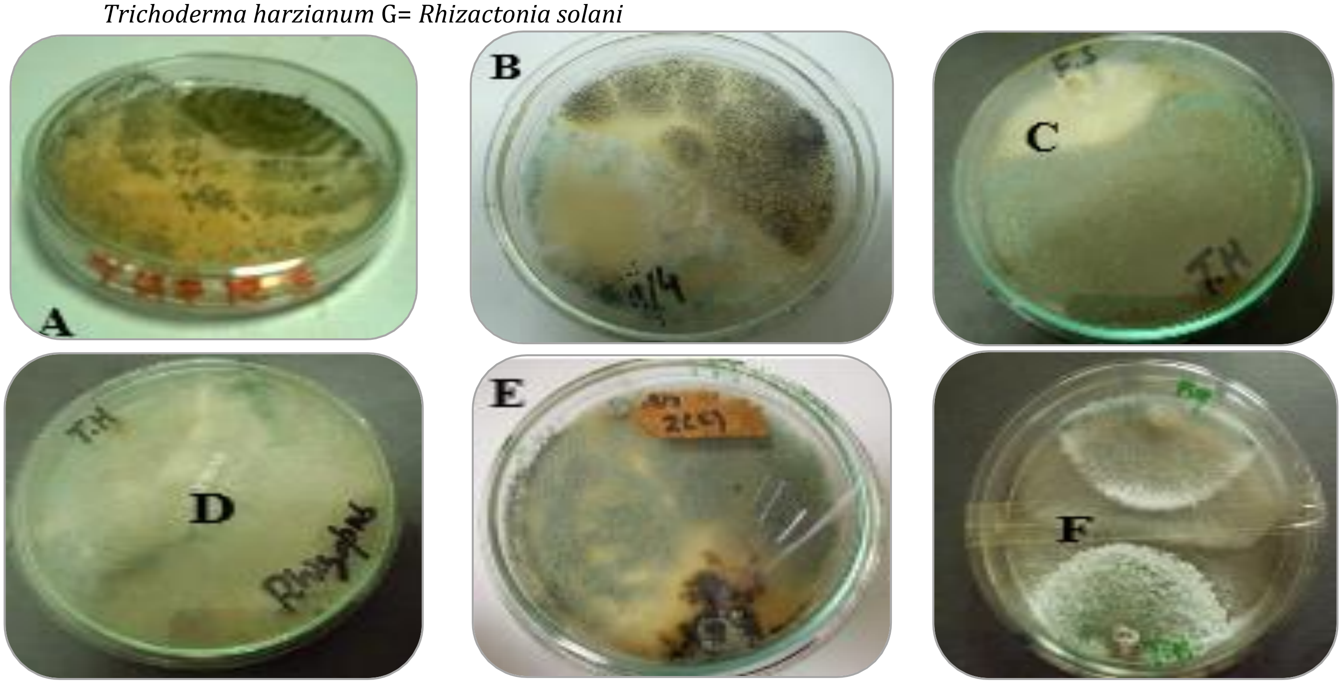

Figure 2. (A) = *T. harzinaum+R. solani* (B) = *T. harzianum+A. nigar* (C) = *T. harzianum+F. solani* (D) =*T. harzianum+R. oryzae* (E) =*T. harzianum+ A. solani* F= *T. harzianum+P. infestans*

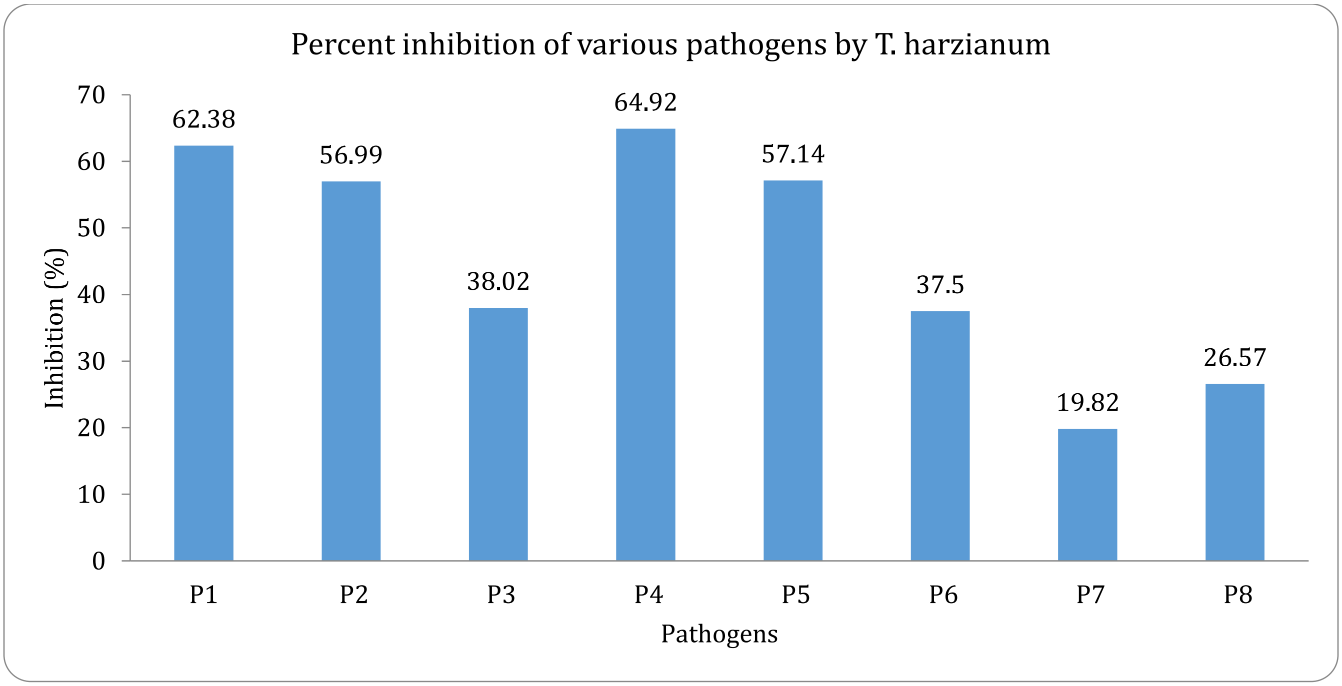

Figure 3. P1= *Fusarium solani,* P2= *Alternaria triticina,* P3= *Aspergillus niger* P4= *Phytophthora infestans,* P5= *Sclerotium rolfsii,* P6= *Bipolaris* P7= *Rhyzopus oryzae,* P8= *Rhizactonia solani*

Table 2. *In vitro* interaction of *Trichoderma harzianum* with microorganism in dual nature

Sr. No	*Fungi*	Days of incubation	Radius of colony (cm)		Types of interaction
			Antagonist	Pathogen	
1	*Fusarium solani*	7	3.4	2.6	C
2	*Alternaria spp*	7	5.9	2.8	A
3	*Aspergillus niger*	7	4.8	3.8	C
4	*Phytophthora infestan*	7	3.3	2.4	C
5	*Sclerotium rolfsii*	7	5.5	3.0	F
6	*Bipolaris*	7	2.9	0.9	C
7	*Rhyzopus oryzae*	7	5.9	5.9	E
8	*Rhizactonia solani*	7	8.8	5.5	B

A = Antagonist completely overgrew on entire medium.

B= medium was completely covered with pathogen while zone formation also occurred.

C= Zone of inhibition is formed.

D= the pathogen grows more than the antagonist and zone formation also occurred.

E= both pathogen and antagonist colonize equally on medium and neither pathogen nor antagonist dominate each other.

F= most of the medium surface was colonized by pathogen.

DISCUSSION

Wheat is an important cereal crop which is affected by various wheat borne fungal pathogens. These wheat-borne pathogens affect yield of a crop which results in economy decline. Chemical pesticides are used to control these wheat pathogens but this is not an ideal alternative. Because of use of such chemicals many other problems arise. In recent year, Biological control instead of using chemical treatment has proved to be more effective (Elad, 2000; Howell, 2003).

In present work, results were collected after biological control of selected pathogenic fungi that were previously isolated from wheat plants on Potato Dextrose Agar medium that were later tested with *Trichoderma harzianum* to find the efficacy of antagonist.

Trichoderma harzianum is extensively used on a variety of plants in farming. These act as bioprotectants, biofertilizers, biostimulants, and bio pesticides (Harman *et al*., 2004) to control foliar, soil borne and post-harvest plant fungal pathogens. Sharma *et al*. (2009) have studied *Trichoderma* efficacy on the plants. *Trichoderma viride* and *T. harzianum* were reported by a number of workers as the most excellent antagonists for growth inhibition of various soil and seed borne plant pathogens.

In under study experiment, *Trichoderma harzianum* inhibit the growth of *Alternaria triticina* by 56.99%, while growth of pathogen was inhibited by 67.07% with the application of *T. harzianum in vitro*. In contrast, Howell (2003) reported that by using different mechanism, *Rhizoctonia solani, Macrophomina phaseolina* and *Rhizopus oryzae* were controlled by *Trichoderma viride* and was studied. In present study *in vitro* control of pathogens was achieved.

Trichoderma harzianum was considered as potential biological control agent of *Alternaria.* In all environmental conditions control of wheat borne pathogen was almost similar when analyzed by Sempere and Santamarina (2007). Patale and Mukadam (2011) observed that antagonistic actions was exhibited by many *Trichoderma* species to control diseases caused by following pathogens, *Aspergillus flavus, Alternaria solani, A. niger, Phytophthora* sp.*, F. oxysporum, R. solani,* and *Penicillium notatum*.

Ambuse *et al.* (2012) investigated that many species of *Trichoderma* performed antagonistic affect but most importantly *T. viride, T. koningii and T. pseudokoningii* are species that show significant inhibition of *Alternaria tenuissima*. The activity shown by *Trichoderma sp.* was 80% to control the diseases caused by *A. tenuissima*.

During the present study, *Trichoderma harzianum* control the growth of *Fusarium solani* by 62.38% but Hibar *et al*. (2005) found that the antagonism *in vitro* of *Trichoderma harzianum* against *Fusarium oxysporium* showed that inhibition of the pathogenic fungus growth was more than 65%, furthermore, the volatile metabolism substances released from *T. harzianum* decreases the plant pathogenic fungus growth by 63% when compared with controls. *Trichoderma harzianum* showed strong antagonism against fungal species *Aspergillus niger, Fusarium solani,* and *Rhizopus*. The similar occurrence was observed by (Sallam *et al*., 2008; Jegathambigai *et al*., 2009).

Under study experiment, *Trichoderma harzianum* show maximum inhibition in the growth of *Sclerotium rolfsii* (57.14%) however isolates of *Trichoderma* differed in their antagonistic activity against *Sclerotium rolfsii (*Elad *et al.,* 1980; Maity and Sen, 1985). During dual culture plate assay between *Trichoderma harzianum* and *Phytophthora infestans,* antagonist show maximum growth reduction of pathogen. In current study, growth of *P. Infestans* was retarded by *T. harzianum.* The antagonistic actions shown by *Trichoderma* are due to the production of enzymes that degrade cell wall of pathogenic fungi and also produce antibiotics (Sharma *et al.,* 2009). The observed mycoparasitic action shows that *Trichoderma* has greater potential to control *P. Infestans* that cause late blight disease in potato. Similar action was reported to control pathogens, *Phytophthora capsici* and *Phytophthora cinnamomi* (Ezziyyani *et al.,* 2007).

In current study, *In vitro* effect of *Trichoderma harzianum* on *Bipolaris* show inhibition of 37.50% while according to the Berber *et al*. (2009) when studying the effect of the antagonism *in vitro* between six isolates of the antagonistic fungus *Trichoderma harzianum* against four pathogenic isolates of *Bipolaris* and found that the *Trichoderma harzianum* inhibited the pathogenic fungus growth with a different rations, including the following: 68.55-72% and 69.52-73.32% for each of *B. maydis* and *B. sorghicola* respectively, and 67.02-70.02% for each of *B. sorokiniana* and *B. tetramra.*

In our investigation, *Trichoderma harzianum* show strong antagonism against *Fusarium solani* (62.38%). Tawfik and Allam (2004) exposed that the *Trichoderma sp* isolates show strong antagonism against wilt diseases that are caused by *Fusarium*, *in vitro*, on potato dextrose medium, reduce the *Fusarium* growth with the following proportions: 88%, 80% and 86% for three antagonistic species including *Trichoderma harzianum*, *T. viride* and *T. hamatum* respectively. The antagonist *Trichoderma harzianum*, *T. coningi* and *T. viride* were proved to be equally antagonistic to *F. udum* under *in vitro* conditions.

Sivan and Chet (1989) observed that *Trichoderma* spp. effectively control *Fusarium* spp. that causes diseases on cotton, wheat and muskmelon. They investigated that *Trichoderma* species are more common occupant of rhizosphere and play important role to control a lot of soil borne plant pathogens that increase plant diseases.

In present study, *Trichoderma harzianum* significantly inhibited a number of plant pathogens (Dubey, 2003). It is very significant to note that *Trichoderma* species are famous to produce a range of antibiotics such as trichodernin, trichodermol and harzianolide (Dennis and Webster, 1971; Howell, 2003; Kucuk and Kivanc, 2004). In addition to some cell wall degrading enzymes such as glucanase and chitinase which break down the polysaccharides and chitins, thereby increase the cell wall integrity (Elad *et al.,* 1983; Elad, 2000).

Comparing the findings of this study with previous research reveals some similarities and differences. In general, the results align with earlier studies that demonstrated the negative environmental impact of conventional farming practices and the benefits of adopting sustainable alternatives. However, the specific effects on soil quality, biodiversity, and ecosystem services may vary depending on factors such as regional climate, soil types, and local agricultural practices.

The potential implications of this study's findings for agriculture and the environment in terms of biological control are noteworthy when considered alongside previous research. The observed increase in crop yields due to the use of biological control agents, such as *Trichoderma harzianum*, can lead to reduced land use and improved food security, which aligns with the findings of Hibar *et al*. (2005). However, the study's findings on water use efficiency and soil health highlight the need for more research on the long-term effects of biological control methods. While some studies have reported contrasting results regarding soil health and microbial activity, the present study emphasizes the importance of further research in this area. Lastly, the reduction in pesticide use observed in crops with biological control agents has positive environmental implications, which is consistent with findings of other studies (Perry *et al*., 2016). These results should be considered in tandem with existing research to better understand the complex relationships between biological control, agriculture, and the environment.

One notable difference in the current study may be the extent to which specific sustainable practices contribute to improving environmental outcomes. This can be attributed to advances in research methodologies, increased awareness of the potential benefits of sustainable agriculture, and the availability of new technologies that facilitate the implementation of these practices. Overall, the findings of this study contribute to a growing body of evidence supporting the adoption of sustainable agricultural practices for the benefit of both agriculture and the environment.

Although, additional work is required to understand the mechanism of *Trichoderma harzianum* and how the antagonists strains are improved and development of further products that are used as biological control agent to control plant pathogens. Therefore, it is obvious that microorganism's used as biological control agents are risk-free not only to human beings but also for animals. Those products that are formed to control pathogens are cheaper to use than fungicides and are highly successful. It is now extensively recognized that use of environmentally friendly bio pesticide is a diverse possibility in the future and these can be successfully applied in modern agriculture especially within the framework of integrated pest management system without affecting our valuable ecosystem. There is nothing in biological control that should be thought of as unfeasible at the present condition of our technology.

Significance of the Study: The study highlights the effectiveness of *Trichoderma harzianum* as a biological control agent against various wheat-borne fungal pathogens, demonstrating the potential of biological control as a viable alternative to chemical pesticides, which have been shown to harm the environment. By identifying *Trichoderma harzianum* as an effective antagonist that can significantly inhibit the growth of various wheat-borne pathogens, the study suggests that it could be a promising candidate for biological control of these pathogens in wheat crops. Additionally, the results support the development of environmentally friendly alternatives to chemical pesticides, potentially reducing the negative impacts of chemical pesticides on the environment, human health, and non-target organisms. Furthermore, the findings serve as a foundation for further research on the use of *Trichoderma harzianum* as a biocontrol agent. Future research could focus on optimizing its application, studying its interactions with other biological control agents, or investigating its potential for use in integrated pest management strategies. In conclusion, the study provides significant insights into the potential of *Trichoderma harzianum* as a biological control agent against wheat-borne pathogens and contributes to the ongoing search for sustainable, environmentally friendly alternatives to chemical pesticides.

Limitations of the Study: While the study offers valuable insights into the potential of *Trichoderma harzianum* as a biological control agent against wheat-borne pathogens, some limitations warrant further investigation to strengthen the evidence base and inform practical applications in agriculture.

Focus on specific population: The study concentrated on a particular demographic or geographic area, which allowed for an in-depth exploration of the research question within that context. However, this also limits the generalizability of the findings to other populations.

Use of specific assessment tools: The study employed particular assessment tools, which enabled a targeted analysis of the variables of interest. Nonetheless, this may have excluded other relevant aspects that alternative instruments could have captured.

Limited scope of investigation: The study's scope was intentionally narrowed to focus on specific variables, enabling a more detailed examination of their interrelationships. This deliberate limitation, however, leaves room for future research to explore other aspects or factors not addressed in this study.

Time constraints: Due to time limitations, the study utilized a cross-sectional design, which allowed for a more efficient data collection process. While this design has its limitations in establishing causality, it still provides valuable information on the associations between variables at a specific point in time.

Resource limitations: The study was conducted with limited resources, which necessitated the use of a smaller sample size and simpler research design. Despite these constraints, the study still offers valuable insights and can serve as a foundation for future research with more extensive resources.

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CONFLICT OF INTEREST

The author declared that she has no conflict of interest.

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Contribution of Authors:

Ayesha Bibi	:	Study conception, design, material preparation, data collection and the first draft of the manuscript
Najam-ul- Sehar Afshan	:	Final corrections in the manuscript
Fouzia Yaqoub	:	Methodology; Supervision