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ANTAGONISTIC SCREENING AND CONFRONTING POTENTIAL OF *TRICHODERMA VIRIDE* AGAINST PAKISTANI AND AMERICAN SOIL BORNE-PATHOGENS (*PYTHIUM APHENIDERMATUM*, *FUSARIUM OXYSPORUM* AND *PHYTOPHTHORA CAPSICI*) IN CONTROLLED CONDITIONS

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ABSTRACT

The study's major goal was to find more efficient antagonist fungus for soil-borne diseases of chilli and tomato plants. *Trichoderma* species (*T.asperellum* and *T.viride*) were investigated for antifungal potential against Pakistani and American soil-borne pathogenic isolates of *Pythium aphendermatum, P. capsici, P. infestant*, and *F. oxysporum*. In-vivo conformation aggressiveness was tested using *Trichoderma* strains that had been identified morphologically and molecularly. Both biocontrol agents (*T.asperellum* and *T.viride*) were found effective to suppress the pathogenic *Fusarium oxysporum* isolate; *T.viride*, on the other hand, suppressed *Phytophthora* spp. (40%) and *T.asperellum* reduced growth up to 70% of *Pythium aphendermatum* isolates from the United States of America. *T.viride* and *T.asperellum* showed similar degrees of inhibition against pathogenic fungi (*Pythium, Phytophthora*, and *Fusarium*) of Pakistani isolates (60-90%). Both BCA fungi were treated simultaneously and maximum inhibition was observed up to 90%. Whereas *T. asperllum* was found more efficient than *T. viride* to inhibit the infection by *Pythium, Phytophthora*, and *Fusarium* species. When antagonistic fungi were coupled, the *Trichoderma* strain demonstrated broad spectrum antagonism effect, which is a critical characteristic for the economic sustainability of any biocontrol agent.

Keywords: Confrontation, Dual culture, Biocontrol fungi, Antifungal potential.

INTRODUCTION

The most prevalent biological control agents (BCAs) of the ascomycete genus *Trichoderma* are soil-based and have been documented to utilize a variety of biocontrol strategies. For their practical implementation in agriculture, it is necessary to explore the synergistic effects produced by diverse genotypes. In the fight against a wide range of diseases Inhibition by antimicrobial chemicals (antibiosis), competition for colonization sites and feeding, degradation of pathogenicity factors, and parasitism are all examples of antibiotic substances produced by *Trichoderma* (Sivasithamparam and Ghisalberti, 1998). Indirect solutions include improved plant nutrition and damage compensation (Kumar, 2013). Antibiosis is caused by interactions between pathogenic microbes and low-molecular-weight diffusible chemicals, or antibiotics generated by *Trichoderma* strains. Trichoderma also produces antibiotic chemicals that aid in the destruction of plant pathogens, as well as extracellular enzymes that may help *Trichoderma* maintain a healthy biota balance (Bentez *et al*., 2004: Harman *et al*., 2004). *Trichoderma* species grow quickly (Howell, 2002) and fight for nutrients and space with soil microbes (Elad, 1996).

PCR for the ITS region was used to identify the genomic variability of isolates (AL-Abedy *et al*., 2020).

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phylogenetically different species were characterized as *T. harzianum, T. hamatum, T. longibrachiatum, T. asperellum, T. atroviride, T. koningi,* causes infection in mashroom as a green mold disease. *Trichoderma* can be found all over the world. Different species of *Trichoderma* have been found to be more prevalent in cultivated to non-cultivated, tropical to highly elevated areas, and deadly to healthy plants in Russia, Nepal, India, Tunisia, Peru, Ecuador, France, Italy, Rawanda, South Africa, Romania, and Guatemala due to its importance as a biopesticide (Jaklitsch *et al*., 2006; Anees *et al*., 2010; Kulling *et al*., 2000; Sadfi-zouaoui *et al*., 2009; AL-Abedy *et al*., 2020).

Trichoderma has become a popular biocontrol agent as a natural alternative to synthetic pesticides, and it was utilized to confirm a link between molecular and morphological characteristics and antagonistic activity. The antagonistic effects of *T. harzianum* and *T. viride* on *F. oxysporum* f.sp. *lycopearsici* and *X. campestris* pv. *vesicatoria* were investigated. *T. harzianum* isolates were more aggressive than *T. viride* isolates. The sporulating structures of *Trichoderma* isolates were compared and morphologically characterized on colony morphology (Iqbal *et al.,* 2017). The potential ability of *Trichoderma* isolates to suppress the pathogen was revealed using a dual culture method. Synthetic fungicides have a wide range of effects on fungal resistance, ecosystem imbalance due to residue harmful effects, human and animal health concerns, as well as the quality and quantity of produce (Johnson and Atallah, 2006). For the management of fungal infections of plants infecting the chilli and tomato crops, researchers are now attempting to convert to non-synthetic bio-fungicides. (Akhtar *et al.,* 2017). The use of antagonistic microorganisms as plant disease biocontrol agents can effectively solve these concerns. (Cook and Baker, 1983).

Chemical therapies for root diseases spread by soil are exceedingly dangerous, posing environmental and economic risks. As a result of the rising interest in environmental preservation, scientists are examining the use of biological agents that can provide effective long-term protection while having no harmful influence on the environment or human health. *Trichoderma* strains are a fascinating alternative to well-known fungicides that are utilized as a plant growth promoter and pathogen antagonist. It can also help with long-term sickness prevention and environmental risk reduction activities.

Growing demand in agriculture for environmentally friendly disease control options, effective identification of *Trichoderma* isolates in combat with various plant harmful fungus. To find a superior strain of *Trichoderma* for commercial usage, a combined morphological and molecular gene technique can be applied. Only when the biocontrol agent appropriately regulates the connection between the host plant and pathogen can a biocontrol program be developed. *Trichoderma* can successfully navigate this combination of plant defensive reactions. As a result, employing *Trichoderma* as a biocontrol agent would almost certainly ensure long-term disease control.

MATERIALS AND METHODS

Sample collection: Plants and soil samples were collected from different vegetables fields of Rawalpindi, Chakwal, Faisalabad and Multan districts of Punjab province.

Samples were collected from various fields of Okra, chilli, brinjal, pumpkin, coriander, mint, garlic, bitter gourd, cabbage, tomato, potato carrot, and squash in several towns of Punjab, Pakistan were visited, and samples were taken by removing 2-3cm of surface soil near the root zone. Plants and soil samples that appeared to be healthy were appropriately labelled and covered in polythene bags. Symptomatic probable diseased plants, plant parts (leaves, fruits, roots, stems), and soil (from the infected plant's root zone region) infested with *Fusrium, Pythium*, and *Phytophthora* spp. were collected in airtight zipper lock bags and tagged with the date, location, and host plant. All the collected samples were placed in ice bucket and brought to the biotechnology lab at CABI Rawalpindi. Plant based samples like leaves, fruits, roots, stems initially rinsed with tap water for the removal of superficial contamination and preserved at 4^{o}C for further isolation of *Trichoderma, Fusarium*, *Pythium* and *Phytophthora* spp.

Isolation and identification: Isolation of *Trichoderma* species

The multiple tube dilution technique (MTDT) was used for the isolation of *Trichoderma* sp. (Rahman *et al.,* 2011; Samson *et al.,* 2010). 1gm of soil was dissolved in 10ml distilled water that had been sterilized and vortexed for 1 minute, after which 1ml of the supernatant was suspended in 9ml sterilized water to achieve 10-fold dilutions (10^{-1} to 10^{-9}). 1ml of each of the 10^{-3} to 10^{-5} concentrations were distributed/streaked on the petri

dishes. Plates were adequately wrapped in parafilm and kept in the incubator for 4-7 days at a temperature of 26°C. A mixed growth of numerous fungal and bacterial cultures were seen on the inoculated plate. After that, the desired green fungal colonies were selected and plated on new PDA plates. These colonies were further purified using streaking or single-spore methods. Pure inoculated plates were cultured and incubated for another 7-8 days at 26°C. A spade was used to spread 1 ml of distilled water over the fully grown *Trichoderma* culture, it was then suspended in 99ml of autoclaved distilled water. Under the microscope at 10-40X magnification, a drop of fungal suspension was stained with lactophenol and identified according to Nelson *et al.* (1983).

Isolation of *Fusarium, Pythium* and *Phytophthora* Spp.: Different methods were adopted for the isolation of *Fusarium*, *Pythium* and *Phytophthora* spp.

I. Direct sample plating:

Wilted tomato plant tissues (stems, leaves, and roots) were collected from a farmer's field, washed, and cut into little pieces alongside healthy portions. Surface sterilization was performed using a 5% NAOCl solution for 5- 10 minutes, followed by three washings. Drying of samples was done in autoclaved filter paper after sterilization and washing. After that, samples were cultured for 3-5 days on potato dextrose agar (PDA) medium and incubated at 25 ± 30^{0}C.

II. Baiting technique for isolation of *Pythium* spp.

Pythium spp. were isolated using the Baiting approach from contaminated soil samples taken from the root zone of diseased chilli and tomato plants. Sterilized water was used to make the soil paste. On one side of the petri plate, one teaspoon of soil paste was deposited. Following that, 10 mL autoclaved distilled water was carefully poured into petri dishes, and sterilized dicot grass blades were carefully placed in petri dishes with one end of the leaf blade touching the soil and two others near the first, as shown in Fig 1. To enhance mycelial growth on grass blades, incubation at room temperature (22-25°C) for 5-8 days is recommended. Coenocytic mycelial threads were collected and gently transferred to water agar or corn meal agar (CMA – PARP) media. Inoculated petri plates were incubated at 25°C for 3-5 days for oomycetes growth and purification (Lodhi *et al.,* 2013).

III.Cucumber and carrot fruit bait

As illustrated in Figure 2, a portion of infected soil was placed in a polyethylene bag, moistened with sterilized autoclave water, and a slice of cucumber was positioned in such a way that one end of the cucumber touched the soil. The zipper bags were then incubated at a temperature of 25 ± 2^{o}C. On cucumber fruit, small amounts of coenocytic hyphae were visible after 3-5 days of incubation. To eliminate debris, whitish mycelial partners were rinsed with water and transferred onto CMA–PARP modified media. For culture fungal agents, plates were incubated at 25 ± 2^{o}C once more (Akhtar *et al.,* 2017; Akgul *et al.,* 2008; Lamour *et al.,* 2012)

Serial dilution technique: Fungi were isolated using a multiple plate dilution approach (Fred and Wakesman,1922). 1gm of infected soil, serial dilutions (10-1 to 10-9) were generated using this approach. Following the fabrication of serial dilutions, one ml aliquot sample from each dilution (10^{-5} to 10^{-7}) was obtained and disseminated over CMA-PARP and PDA media using a sterile rod, followed by incubation at 25^{0}C ± 2 for growth.

In-vitro antifungal potential of *Trichoderma viride* and *Trichoderma asperellum* against Pakistani and American soil borne fungal pathogens (*Pythium aphenidermatum*, *Fusarium oxysporum* and *Phytophthora capsici*)

The antifungal activity of *Trichoderma* sp. against American and Pakistani fungal isolates was evaluated using dual culture of pathogenic fungus (*Pythium aphenidermatum, Fusarium oxysporum, Phytophthora infestant,* and *Phytophthora capsici*) and bio-control (*T.viride* and *T.asperellum*) agents. The margins of mother cultures yielded two plugs of actively growing mycelium, one carrying *Trichoderma* stock (*T. viride* and *T.asperllum*) and the other harbouring soil-borne pathogens (*Pythium, Fusarium* and *Phytophthroa* spp). A plug of fungal pathogen (*Pythium, Fusarium,* and *Phytophthroa*) was kept as a control. Inoculated cultured plated were incubated for 4-5 days at 25°C in three repetitions. For 3-5 days, the radius of the colony was calculated at regular intervals of 24 hours. Zone and percentage of inhibition was measured via the principle:

$$L = \frac{(C - T)}{C} \times 100$$

Whereas L: Radial mycellular growth inhibition; C: Radial pathogen development in control; T: Radial pathogen development in the presence of *Trichoderma viride* and *T.asperellum* (Fadwa *et al.,* 2009; Mokhtar and Aid, 2013).

RESULTS AND DISCUSSION

The purpose of this work was to isolate, identify, and describe the biocontrol fungus *Trichoderma* as a more effective biocontrol agent against *Pythium, Phytophthora*, and *Fusarium*, which cause sickness in chilli and tomato crops. These biocontrol fungi are environmentally friendly and cost-effective since they may live for years in any type of soil and under adverse conditions. The similar conclusion was reached by Gohel *et al.* (2007) about its ability to thrive in hostile conditions.

Isolation of *Trichoderma* and Pathogenic Fungi: More than 200 *Trichoderma* isolates were isolated from vegetable fields in Punjab, Pakistan (okra, chilies, brinjal/eggplant, pumpkin, coriander, mint, garlic, bitter gourd, cabbage, tomatoes, potatoes, carrots, squash), while pathogenic fungi were isolated from infected soil and plant parts of tomato and chilli crops. Following purification on PDA, morphologically different colonies were chosen based on their morphological traits (Kubicek and Harman, 1998). *Trichoderma, Fusarium, Pythium*, and *Phytophtora* were isolated using a variety of techniques from healthy as well as diseased disease plant sections and infected soil. PDA was shown to be the best medium for the isolation of *Fusarium* and biocontrol fungus (*Trichoderma* Spp) while CMA-PARP was the best for *Pythium* and *Phytophthora* spp. isolation.

Figure 1. Baiting Technique for the isolation of oomycetic fungi (*Pythium*).

Figure 2. Cucumber and carrot fruit bait for isolation of *Pythium* and *Phytophthora.*

Isolation of pathogens: Various approaches were used to isolate *Pythium, Phytophthora*, and *Fusarium* species. Baiting and serial dilution techniques were utilized to isolate pathogenic fungus, and pure cultures of pathogenic fungi were identified using a compound microscope. *Pythium aphenidermatum* is an Oomycete that belongs to the *Peronosprales* order. It has a wide host range and prefers warm temperatures, which is why it is commonly found in greenhouse plants. It infects plants by motile zoospore, and damp conditions aid disease transmission in seedling plants. Under a compound microscope, the hyphae are hyaline and the mycelium has no cross walls. Sporangia, oogonia, and antheridia were also discovered under the microscope. Sporangia are easily visible as asexual lobate (inflated) spores during morphological examination. The antheridia's intercellular (rarely terminal) attachment and the apluerotic oogonium (oospore does not fill the oogonium) are shown in Fig 3. (a).

Under a compound microscope, *Phytophtora capsici* and *Phytophtora infestant* were identified. *Phytophthora capsici*, often known as water mold fungus, produce millions of short-lived, lemon-shaped motile spores under warm and humid weather, as illustrated in Figure 3. (b).

Fusarium oxysporum is an ascomycete fungus that creates a delicate white to pink or purple tinge on PDA cultivation, according to morphological observation. Morphological traits such as the development of three types of spores: microconidia, macroconidia, and chlamydospores, confirmed the existence of *Fusarium oxysporum*. According to morphological studies, microconidia are plentiful, oval-ellipsoid, straight to curve. non-septated and are borne on simple phialides erupting laterally. The macroconidia are thin-walled, three- to five-septate, fusoid-subulate, and pointed at both ends, with a pedicellate base, and are borne on branched conidiophores or sporodochia's surface. The most common spores are three-septate spores. There are many smooth and rough-walled chlamydospores that form terminally or intercalarily. They are normally single; however, they can occasionally form pairs or chains. Figure 3(c) shows macroconidia.

Oospores, which are sexual reproductive spores with thick walls and a globose shape, are produced by *Phytophthora infestant*. It developed asexually thick walled chlamydospores when the conditions were adverse. It can germinate sporangiophores containing sporangia under ideal conditions. Sporangia are single-celled flagellated zoospores that can swim through a thin coating of water as shown in Figure 3(d).

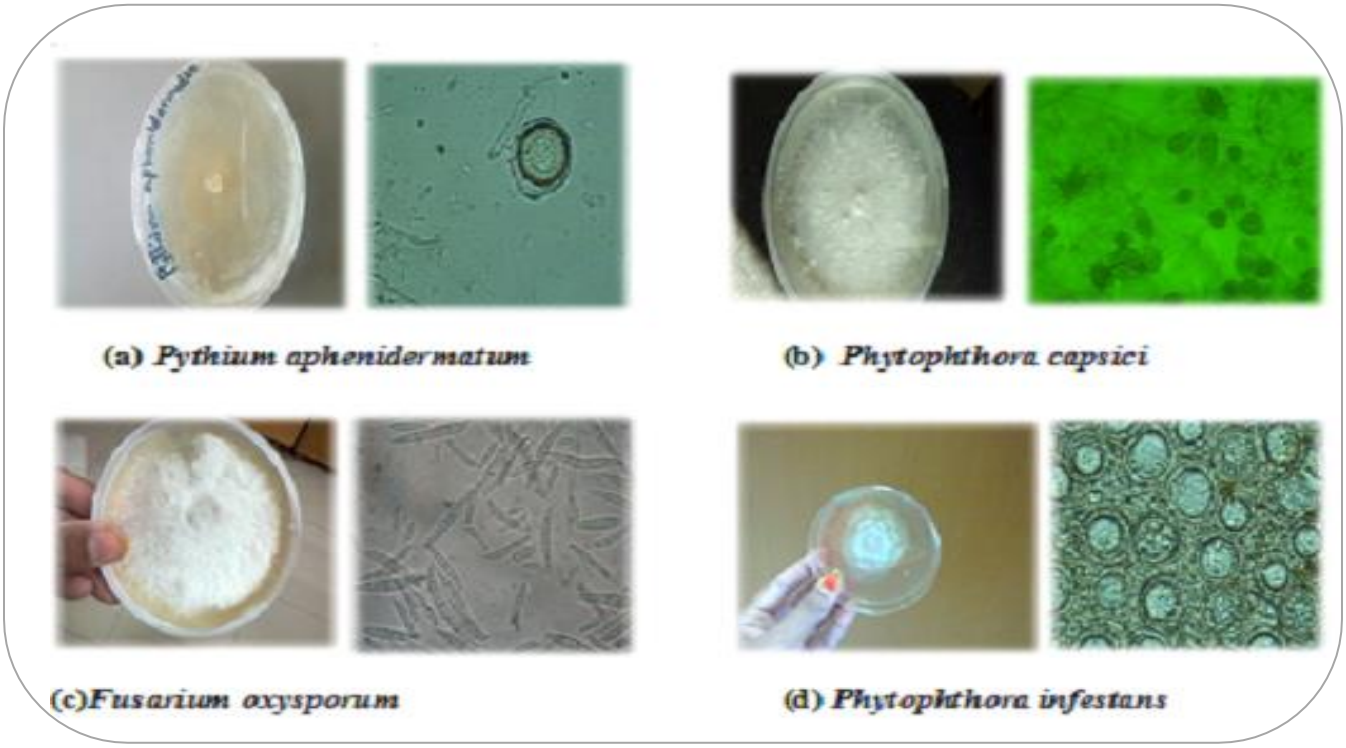

Figure 3. Pure culture of isolated plant pathogens from chilli and tomato crop and soil (a-d)

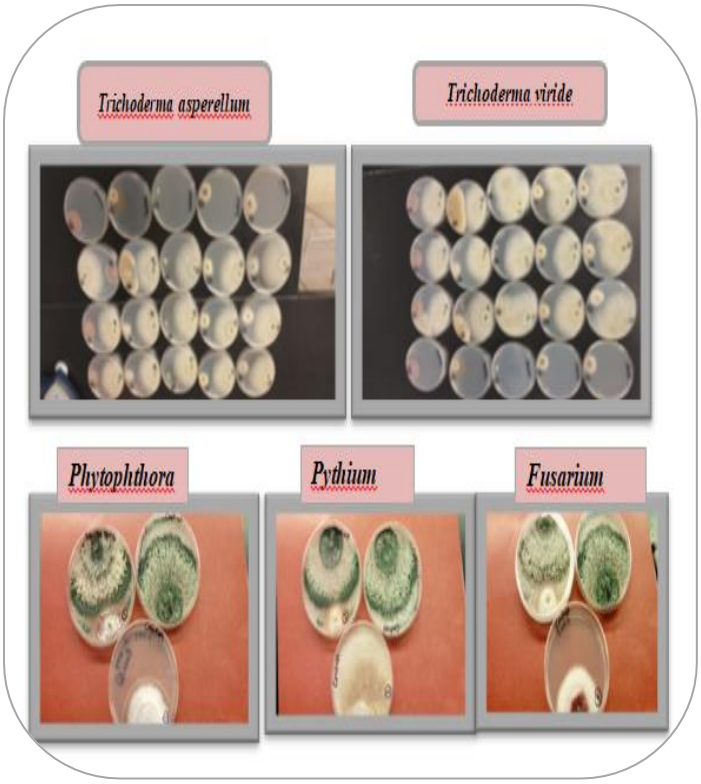

Figure 4. Antifungal potential of Trichoderma isolates against
against *Pythium, Phytophthora* and *Fusarium* of Pakistani and American isolates

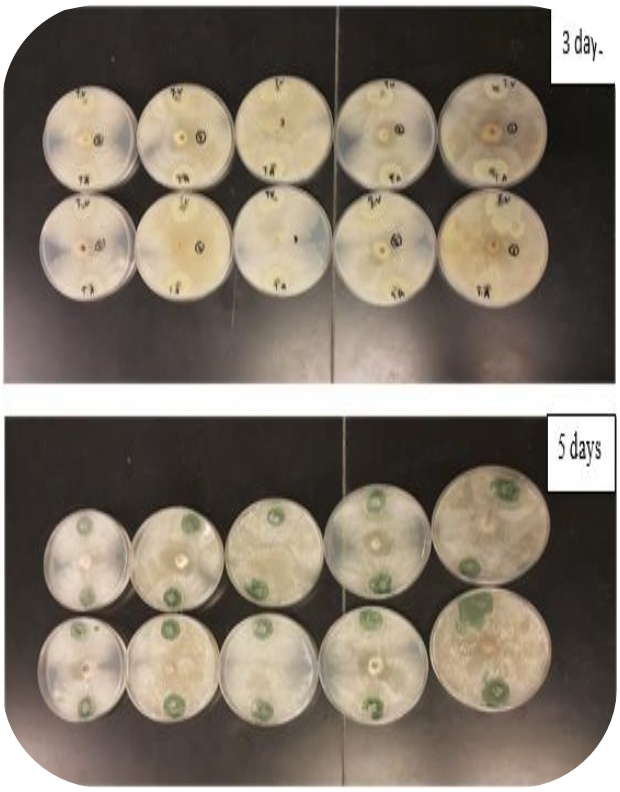

Figure 5. Combine effect of T.viride and T.asperellum pathogenic fungi

In-vitro antifungal potential of *Trichoderma viride* and *Trichoderma asperellum* against Pakistani and American soil borne fungal pathogens (*Pythium aphenidermatum, Fusarium oxysporum* and *Phytophthora capsici*)

With intervals of 24 hours, 48 hours, and 72 hours, the antifungal ability of *T. viride* and *T. asperellum* was examined for American and Pakistani isolates of *Pythium, Pytophthora*, and *Fusarium*. In the presence of biocontrol fungus, the results of direct confrontation of *T. viride* and *T.asperellum* against *Pythium, Phytophthora*, and *Fusarium* of American isolates revealed decreased hyphal growth of American pathogenic isolates, as shown in Figure 4. Table 1 shows that *T.viride* inhibited *Phytophthora* and *T.asperellum* inhibited *Pythium* sp by 40-70 percent, respectively, in the case of *Fusarium* isolate, inhibition by T.*viride* and T.*asperellum* was same. *Trichoderma* sp. has a well-known strategy for controlling other fungi: competition for space and nourishment. The dual culture technique, which involves plating both fungi on agar media, makes it simple to study (Al-Saeedi *et al.,* 2014; Harman, 2000). *Trichoderma* specie can compete directly with phytopathogens for space and resources by creating metabolic and antibiotic chemicals that prevent spore development (Tapwal *et al.,* 2011; Naher *et al.,* 2014). *Trichoderma* is naturally pesticide resistant, allowing it to develop quickly.

Table 1. Mycelial growth of soil borne American isolates in the presence of selected *Trichoderma* spp

Treatments	Mycelial growth of pathogenic fungi			
	Pythium	*Phytophthora*	*Fusarium*	Mean
T.viride	1.24a	0.273c	0.577bc	0.691A
T.asperellum	0.561bc	0.627b	0.735b	0.6415A
Means	0.902A	0.450B	0.646B	
Control	2.112A	1.45B	1.26C	
	LSD: 0.05 BAC: 0.169 BCA*Treatment: 0.292 Treatment: 0.207			

As shown in Table 2, *T.viride* and *T.asperellum* had the same amount of inhibition (60-90%) against harmful fungus (Pythium, Phytophthora, and Fusarium) of Pakistani isolates. When both BCA fungi were employed together against these pathogenic isolates, the level of inhibition was significantly higher than when they were treated separately. Overall, *Trichoderma viride* is more effective in controlling *Pythium, Phytophthora*, and *Fusarium* than *Trichoderma asperllum*, with a 70-90 percent inhibition rate, as shown in Figure 5 and Table 3. Fadwa *et al.* (2009) observed that *T. harzianum* and *T. viride* influence the growth and spore generation of the Bipolaris fungus. When compared to control, the degree of parasitism of *T. viride* and *T.asperellum* in Pakistani and American isolates of *Pythium*, *Phytophthora*, and *Fusarium* suppressed mycelial growth by more than 65 % (Hibar *et al.,* 2005; Mokhtar Aid, 2012; 2013). It's also been confirmed that *Trichoderma* isolates have a significant level of antagonistic activity. *Trichoderma* isolates also demonstrate a relatively high antagonism mechanism against wilt, root rot, and damping off diseases produced by *Fusarium* sp, *phytophthora* sp, and *pythium* sp, as shown in Tables 4, 5, and 6.

Table 2. Inhibition of soil borne American isolates by selected *Trichoderma* Spp.

Treatments	%Inhibition of mycelial growth			
	Pythium	*Phytophthora*	*Fusarium*	Mean
T.viride	39.911c	79.12a	54.767b	57.934A
T.asperellum	70.296a	42.69c	40.658c	51.217B
Means	55.103AB	60.911A	47.712B	
Control	2.112A	1.45B	1.26C	
LSD: 0.05 BCA: 6.43 BCA*Treatment: 11.11 Treatment: 7.88				

Table 3. Mycelial growth of soil borne Pakistani isolates in the presence of selected *Trichoderma* spp

Treatments	Mycelial growth of pathogenic fungi			
	Pythium	*Phytophthora*	*Fusarium*	Mean
T.viride	0.377c	0.611b	0.544b	0.511B
T.asperellum	0.5378	0.53BC	0.967a	0.6778A
Means	0.457C	0.5706B	0.7551A	
Control	2.112A	1.45B	1.26C	
LSD: 0.05 BAC: 0.089 BCA*Treatment: 0.1552 Treatment: 0.1098				

Table 4. Inhibition of soil borne Pakistani isolates by selected *Trichoderma* Spp.

Treatments	%Inhibition of mycelial growth			
	Pythium	*Phytophthora*	*Fusarium*	Mean
T.viride	69.306a	70.767a	74.006a	71.359A
T.asperellum	61.081b	62.267b	58.136b	60.949B
Means	65.193A	66.571A	66.071A	
Control	2.112A	1.45B	1.26C	
LSD: 0.05 BAC: 4 BCA*Treatment: 6.93 Treatment: 4.902				

Table 5. Compatibility of two *Trichoderma* spp. on mycelial growth of Pakistani soil borne pathogens

Treatments	Mycelial growth of pathogenic fungi			
	Pythium	*Phytophthora*	*Fusarium*	Mean
T.viride +T.asperellum	0.167b	0.335b	0.79a	0.42A
Control	2.04A	1.94A	1.27B	1.68A

Table 6. Antifungal potential of two *Trichdoerma* spp. in inhibition of Pakistani soil borne pathogens

Treatments	%Inhibition of mycelial growth			
	Pythium	*Phytophthora*	*Fusarium*	Mean
T.viride+ T.asperellum	91.68A	85.123A	75.43B	84.09A
Control	2.04A	1.94A	1.27B	1.68A
LSD: 0.05 BAC: 0.00 BCA*Treatment: 0.12 Treatment: 4.902				

Several researchers have reported on the anti-antagonist capability of *Trichoderma* species against various fungal phytopathogens (Papavizas, 1985; Bell *et al.,* 1982; Elad and Kapat, 1999; El-Katatny *et al.,* 2001; Marco *et al.,* 2003; Sanjy *et al.,* 2008; Indira and Kamala, 2011). New strains of *Trichoderma* spp. from the rhizosphere near the host plant, which may display potentially stronger bio controlling power against a spectrum of diseases of the corresponding host plant, must be explored in order to have industrially promising strains. It also exhibits

broad-spectrum antagonistic activity, which is crucial in terms of biocontrol. Trichoderma spp. has been shown to be effective against a wide range of fungal infections in earlier studies (Xiao-Yan *et al.,* 2006; Zivkovic *et al.,* 2010). In-vitro studies to demonstrate the antagonistic potential of *Trichoderma* species, or any other potent antagonist, must consider the number of strains of a particular pathogen in order to determine a range of inhibition, which is critical for validating the efficacy of a specific antagonist against a specific pathogen, according to the current study.

The inhibitory activity of *T. viride* and *T. asperellum* against soil-borne fungal infections revealed in this study was like that discovered in another study (Robert *et al.,* 1993; Dohroo *et al.,* 1999; Abdollahzadeh *et al.,* 2003). During antagonistic inhibition, pathogen and biocontrol fungi compete for space and nutrients. Through antibiosis or mycoparasitism, it also inhibits pathogen development and sporulation.

Trichoderma is a biological regulator that coexists with a number of plant diseases, including *Rhizoctonia cerealis* and *Rhizoctonia solani*, which are soil-borne fungi. (Montealegre *et al.,* 2010; Innocenti *et al.,* 2003). *T. harzianum* and *T.viride* are antagonist fungi that attack the mycelium and produce antibiotics to suppress soil-borne fungal infections (Anees *et al.,* 2010; Hadar and Henis, 1979). *Pythium* and *Phytophthora* diseases are most well-known for causing "damping-off" in tomato and chilli plants. *T. harzianum's* antifungal effectiveness against *Rhizoctonia cerealis* has also been investigated (Rao *et al.,* 2015; Innocenti *et al.,* 2003; Hanson, 2005). *T. harzianum's* antagonist mechanisms include competition for space and nourishment, mycoparasitism, antibiotic (toxin) production, and enzyme production. Mycoparasitism is the parasitism of a fungus (host) by another fungus (mycoparasite). Parasitism can be divided into two categories: necrotrophic (destructive) and biotropic (balanced) (Manocha, 1991). *Trichoderma* spp. are classified as necrotrophic mycoparasites because they produce antibiotics or toxins to kill their hosts (Howell, 2003). In the interaction between *T. virens* and *Rhizoctnia solani*, Weindling. (1941) described the mechanism of mycoparasitism, which involves spiralling around pathogen hyphae, penetration, and subsequent disintegration of the host cytoplasm by producing gliotoxin. (Howell, 2003; Rocha-Ramirez *et al.,* 2002). Later on, gliovirin, a novel antibiotic obtained from *T. virens*, was described as inhibiting the growth of *Pythium ultimatum* and *Phytophthora* species (Howell, 2003). Antimicrobials have also been found in the secondary metabolites of *Trichoderma* species (Vinale *et al.,* 2008).

The experiment used one of the most dependable methods of determining fungal growth rates: colony diameter measurement. Dry weight measurement is likely the most useful tool for monitoring fungal development because there is no required relationship between the spread of a mycelia front on a solid surface and the overall amount of fungus produced (Madan and Thind, 1998). In order to choose efficient indigenous fungal strains for use as biocontrol agents, a deeper understanding of the genetic heterogeneity within *Trichoderma* isolates, as well as their biochemical capabilities, is required. Bioactive compounds of industrial importance, as well as bioactive molecules found in commercial formulations of plant pathogen biological control products, should be investigated further.

REFERENCES

Abdollahzadeh, J., E. M. Goltapeh. and H. Rouhani. 2003. Evaluation of antagonistic effect of *Trichoderma* species in biological control of causal agents of crown and root rot of sunflower (*Sclerotinia minor*) in vitro. Agricultural Sciences Tabriz, 13: 13-23.

Akgül, D., Soner, and M. Mirik, 2008. Biocontrol of *Phytophthora capsici* on pepper plants by *Bacillus megaterium* strains. Journal of Plant Pathology. 90: 29-34.

Akhtar, T., Q. Shakeel, G. Sarwar, S. Muhammad. and Y. Iftikhar. 2017. Evaluation of fungicides and biopesticides for the control of Fusarium wilt of tomato. Pakistan Journal of Botany, 49: 769-774.

AL-Abedy, A.N., R. G. AL-Janabi, Z.A. AL-Tmeme, Alaa T.Salim, and M. Ashfaq. 2020. Molecular characterization of novel isolates of *Rhizoctonia solani*, *Trichoderma atroviride* and *Fusarium* spp. isolated from different plants and cutting woods in Iraq. Pakistan Journal of Botany, 52:1073-1082.

AL-Saeedi, S. S. and B. M. Al-Ani. 2014. Study of antagonistic capability of *Trichoderma harzianum* isolates against some pathogenic soil borne fungi. Agriculture and Biology Journal of North America, 5(1): 15-23.

Anees, M., A. Tronsmo, V.R. Edel-Hermann, L.G. Hjeljord, C. Heraud. and C. Steinberg. 2010. Characterization of field isolates of Trichoderma antagonistic against

Rhizoctonia solani. Fungal Biology, 114: 691-701.

Behzad, H., T. Mousa, R.M. Mohammad, and D. Mahdi. 2008. Biological potential of some Iranian Trichoderma isolates in the control of soil borne plant pathogenic fungi. African Journal of Biotechnology, 7: 967–972.

Bell, D.K., H. D. Wells and C.R. Markham. 1982. In vitro antagonism of Trichoderma spp. against six fungal plant pathogens. Phytopathology, 72: 379–382.

Benítez, T., A. M. Rincón, M. C. Limón and A. C. Codon. 2004. Biocontrol mechanisms of Trichoderma strains. International Microbiology, 7(4): 249-260.

Cook, R. J. and K. F. Baker. 1983. The nature and practice of biological control of plant pathogens. American Psychopathological Society, 1983.

Dohroo, N.P., N.K. Bharat and S. Nayar. 1999. Diseases of spice crops 425-474 Indus Publishing Co., New Delhi, 425-474.

Elad, Y. 1996. Mechanisms involved in the biological control of Botrytis cinerea incited diseases. European Journal of Plant Pathology. 102: 719-732.

Elad, Y., R.D. David, T. Levi, A. Kapat, B. Kirshner, E. Guvrin. and A. Levine. 1999. *Trichoderma harzianum* T39 mechanisms of biocontrol of foliar pathogens.

El-Katatny, M.H., M. Gudelj, K.H. Robra, M.A. Elnaghy, G.M. Gübitz. 2001. Characterization of a chitinase and an endo-β-1,3-glucanase from *Trichoderma harzianum Rifai* T24 involved in control of the phytopathogen *Sclerotium rolfsii*. Applied Microbiology and Biotechnology, 56: 137–143.

Fadwa. B.A.O.T., B. Alain and D. Allal. 2009. Antagonisme in vitro et in vivo de deux Trichoderma à l'egard de quatre éspèces de Bipolaris pathogens sur le sorgho. Bull, Soc. Pharm, Bordeaux. 148: 93-114.

Gohel, V., V. Maisuria. and H.S. Chhatpar. 2007. Utilization of various chitinous sources for production of mycolytic enzymes by Pantoeadispersa in bench-top fermenter. Enzyme and Microbial Technology, 40(160): 8-14.

Hadar, Y., I. Chet and Y. Henis. 1979. Biological control of *Rhizoctonia solani* damping off with wheat bran culture of *Trichoderma harzianum*. Phytopathology, 69: 64-68.

Hanson, J. R. 2005. The chemistry of the bio-control agent, *Trichoderma harzianum*. Science Progress, 88(4): 237-248.

Harman, G. E. 2000. Myths and dogmas of biocontrol changes in perceptions derived from research on Trichoderma harzinum T-22. Plant Disease, 84(4): 377-393.

Harman, G. E., C. R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004. Trichoderma species opportunistic, avirulent plant symbionts. *Nature* Reviews Microbiology, 2(1): 43-56.

Hibar, K., D.R. Mejda, K. Haifa. and E. Mohamed. 2005. Effet inhibiteur in vitro et in vivo du *Trichoderma harzianum* sur *Fusarium oxysporium f. sp. Radicis lycopersici.* Biotechnology, Agronomy and Society and Environment, 9 (5): 163-171.

Howell, C.R. 2002. Cotton seedling preemergence damping-off incited by *Rhizopus oryzae* and Pythium spp. and its biological control with Trichoderma spp. Phytopathology, 92: 177–180.

Indra T.H. and S. Kamala. 2011. Evaluation of indigenous *Trichoderma* isolates from Manipur as biocontrol agent against *Pythium aphanidermatum* on common beans. 3 Biotechnology, 1: 217–215.

Innocenti, G. R. Roberti, M. Montanari and E. Zakrisson. 2003. Efficacy of microorganisms antagonistic to *Rhizoctonia cerealis* and their cell wall degrading enzymatic activities. Mycological Research, 107(4): 421-427.

Iqbal, S., M. Ashfaq, A. H. Malik, I.ul-Hhaq, K. S. Khan and P. Mathews. 2017. Isolation, preservation and revival of *Trichoderma viride* in culture media. J. Entomol. Zool. Stud., 5: 1640-1646.

Jaklitsch, W.M., G.J. Samuels, S.L. Dodd, B.S. Lu. and I.S. Druzhinina. 2006. Hypocrea rufa/*Trichoderma viride*: A reassessment, and description of five closely related species with and without warted conidia. Studies in Mycology, 56: 135-77.

Jeyaseelan, E.C., S. Tharmila. and K. Niranjan. 2014. Antagonistic activity of *Trichoderma* spp. and *Bacillus* spp. against *Pythium aphanidermatum* isolated from tomato damping off. Archives of Applied Science Research, 4: 1623-1627.

Johnson, D.A. and Z.K Atallah. 2006. Timing fungicide applications for managing Sclerotinia stem rot of potato. Plant Disease, 90:755–758.

Kubicek, C. P. and G. E Harman. 1998. Trichoderma and Gliocladium. Basic biology, taxonomy and genetics. Taylor and Francis Ltd. 1.

Kullnig. C., G. Szakacs. and C.P. Kubicek. 2000. Molecular identification of Trichoderma species from Russia, Siberia and the Himalaya. Mycological Research, 104: 1117-25.

Kumar, K., N. Amaresan, S. Bhagat, K. Madhuri. and R.C. Srivastava. 2012. Isolation and characterization of *Trichoderma* spp. for antagonistic activity against Root rot and foliar pathogens. Indian Journal of Microbiology, 52: 137–144.

Kumar. S. 2013. *Trichoderma:* A biological weapon for managing plant diseases and promoting sustainability. International Journal of Agricultural Sciences and Veterinary Medicine, 1(3): 1-18.

Lamour, K. H., R. Stam, J. Jupe and E. Huitema. 2012. The oomycete broad-host-range pathogen *Phytophthora capsici*. Molecular Plant Pathology, 13(4): 329-337.

Lodhi, A. M., M. A. Khanzada, S. Shahzad, A. Ghaffar and C. A. Lévesque. 2013. Prevalence of *Pythium aphanidermatum* in agro-ecosystem of Sindh province of Pakistan. Pakistan Journal of Botany, 45(2): 635-642.

Lyr, H. P.E. Russel, H.W. Dehne, H.D. Sisler. 1998. Modern fungicides and antifungal compounds. H. Hampshire UK: intercept Ltd., 459–467.

Madan, M. and K. S Thind. 1998. Physiology of fungi. APH Publishing. New Dheli.

Manocha, M.S. 1991. Physiology and biochemistry of biotrophic mycoparasitism. In: Arora DK, Rai B, Mukerji KG, Knudsen GR. (eds.) Handbook of applied mycology. Soil in plant Marcel Dekker, 1: 273-300

Marco, J. L. D., M.C. Valadares-Inglis. and C. R. Felix. 2003. Production of hydrolytic enzymes by Trichoderma isolates with antagonistic activity against *Crinipellis perniciosa* the causal agent of witches' broom of cocoa. Brazilian Journal of Microbiology, 34: 33–38.

Matroudi S., M.R. Zamani. and M. Motallebin. 2009. Antagonistic effect of three species of *Trichoderma* sp. on *Sclerotinia sclerotiorum*, the causal agent of canola stem rot. Egyptian Journal of Biology, 11: 37-44.

Mokhtar, H. and D. Aid. 2012. Antagonism capability in vitro of *Trichoderma harzianum* against some pathogenic fungi. Agriculture and Biology Journal of North America, 3(11): 452- 460.

Mokhtar, H. and D. Aid. 2013. Contribution in isolation and identification of some pathogenic fungi from wheat seeds, and evaluation of antagonistic capability of *Trichoderma harzianum* against those isolated fungi in vitro. Agriculture and Biology Journal of North America, 4(2): 145-154.

Montealegre, J., L. Valderrama, S. Sánchez, R. Herrera, X. Besoain, L. M. Pérez 2010. Biological control of *Rhizoctonia solani* in tomatoes with *Trichoderma harzianum* mutants. Electronic Journal of Biotechnology, 13(2): 1-2.

Naher, L., U. K. Yusuf, A. Ismail and K. Hossain. 2014. Trichoderma spp. a biocontrol agent for sustainable management of plant diseases. Pakistan Journal of Botany, 46(4): 1489-1493.

Nelson P.E. and T.A. Toussoun Marasas. 1983. Fusarium species: An illustrated manual for identification. Pennsylvania, USA: Pennsylvania State University Press.

Papavizas, G.C. 1985. Trichoderma and Gliocladium: Biology, Ecology and Potential for Biocontrol. Annual Review of Phytopathology, 23: 923.

Perveen, K. and N.A. Bokhari. 2012. Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*. African Journal of Microbiology Research, 6: 3348–3353.

Rahman, A., M. F. Begum, M. Rahman M. A. Bari, G. N. M. Illias and M. F. Alam. 2011. Isolation and identification of *Trichoderma* species from different habitats and their use for bioconversion of solid waste. Turkish Journal of Biology, 35(2): 183-194.

Rao, K. M., K. S. Raju and H. Ravisankar. 2015. Antifungal properties of native *Trichoderma* isolates against *Sclerotium rolfsii* and *Pythium aphanidermatum* infecting tobacco. Journal of Environmental Biology, 36(6): 1349.

Robert, A. S., S. H. Ellen., and A. N. V. Connie. 1981. Introduction to–food-borne fungi C.B.S, institute of the Royal Netherlands. Academy Arts and Science.

Robert, S., B.J. Zarowitz, E.L. Peterson, and F.R.A.N.C.I.S. Dumler. 1993. Predictability of creatinine clearance estimates in critically ill patients. Critical Care Medicine, 21(10): 1487-1495.

Rocha-Ramírez, V. C. Omero, I. Chet, B. A. Horwitz and A. Herrera-Estrella 2002. *Trichoderma atroviride* G-protein α-subunit gene tga1 is involved in mycoparasitic coiling and conidiation. Eukaryotic Cell, 1(4): 594-605.

Sadfi, Z., C. Najla, R. Sghaier, H. Mustapha, J. Abdel, M. Hajlaoui and A. Boudabous. 2009. Analysis of the diversity of Trichoderma spp. in soil horizons using digested ITS regions. Annals of Microbiology, 59: 459-463.

Sadfi-Zouaoui, N.S.Z., I.H. Hannachi, M.R. Rouaissi, M.R.H.R. Hajlaoui, M.B.R.B. Rubio, E.M. Monte, A.B. Boudabous. and M.R.H.R. Hermosa. 2009. Biodiversity of Trichoderma strains in Tunisia. Canadian Journal of Microbiology, 55: 154-62.

Samson, D., I. A. Apperly, J.J. Braithwaite, B.J. Andrews and S.E. Bodley Scott. 2010. Seeing it their way: evidence for rapid and involuntary computation of what other people see. Human Perception and Performance. Journal of Experimental Psychology, 36(5): 1255.

Samson, R.A and R.E.S. Hoekstra. 1988. Introduction to Food Borne Fungi. Centraalbureau Voor Schimmelcultutes. Netherland.

Sanjay, R., P. Ponmurgan and U.L. Baby. 2008. Evaluation of fungicides and biocontrol agents against grey blight disease of tea in the field. Crop Protection, 27: 689–694.

Sivasithamparam, K. and E. Ghisalberti. 1998. Secondary metabolism in Trichoderma and Gliocladium. In C. P. Kubicek and G. E. Harman (Eds.), *Trichoderma and Gliocladium.* Taylor and Francis, 1: 139-191.

Tapwal, A., U. Singh, G. Singh, S. Garg and R. Kumar. 2011. In vitro antagonism of Trichoderma viride against five phytopathogens. Pest Technology, *5*(1): 59-62.

Vinale, F., K. Sivasithamparam, E. L. Ghisalberti, R. Marra, S. L. Woo and M. Lorito. 2008.Trichoderma–plant–pathogen interactions. Soil Biology and Biochemistry, 40(1): 1-10.

Weindling, R. 1941. Experimental concideration of the mold toxin of Gliocladium and Trichoderma. Phytopathology, 31: 991-1003.

Xiao-Yan, S., S. Qing-Tao, X. Shutao, C. Xiu-lan, S. Cai-Yun. and Z. Yu-Zhong. 2006. Broad-spectrum antimicrobial activity and high stability of Trochokonins from *Trichoderma koningii* SMF2 against plant pathogens. FEMS Microbiology Letters, 260:119–125.

Zivkovic, S., S. Stojanovic, Z. Ivanovic, V. Gavrilovic, T. Popovic. and J. Balaz. 2010. Screening of antagonistic activity of microorganism against *Colletotrichum aculatum* and *Colletotrichum gloeosporoides*. Archives of Biological Sciences, 62: 611–623.

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