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# Antifungal Potential of Biocontrol Agents Against *Phytophthora Capsici* Causing Chili Fruit Rot

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## Abstract

Biocontrol is an environmentally friendly and proficient way to manage the plant diseases which leads to comes true the dream of organic farming. In present study different antagonistic assays (dual culture, volatile and nonvolatile metabolite) were used to investigate the antifungal activity of three already molecular characterized isolates of *Trichoderma viz, Trichoderma asperellum* TH, *Trichoderma harzianum* HM, *Trichoderma harzianum* HK and two morphologically characterized isolates of *Bacillus subtilis* against *Phytophthora capsici* (Leonian) a threating pathogen of fruit rot of chili. Results showed that all the antagonist inhibited the radial growth of tested pathogen. In dual culture assay, *T. asperellum* showed maximum (61.6%) mycelial growth inhibition followed by *Bacillus subtilis* A (54.3%), *T. harzianum* HK (51.4%), *T. harzianum* HM (47.2%) and *Bacillus subtilis* B (41.5%). Culture filtrate (Extracted metabolites/ nonvolatile metabolites) were proved as more efficient inhibitor of pathogen as compared to volatile metabolites. Nonvolatile metabolites of *T. asperellum* TH showed maximum inhibition (44.5%) and minimum inhibition showed by *Bacillus subtilis* B (29.1%) while volatile metabolites of *T. asperellum* TH showed maximum inhibition (28.3%) and *Bacillus subtilis* B (11.5%) gave minimum inhibition as compared to other tested biocontrol agents against fruit rot pathogen. Based on results, it is concluded that biocontrol agents have great potential to manage the *P. capsici* in a better way.

**Keywords:** Chili; Fruit rot; *Phytophthora capsica; Bacillus; Trichoderma;* Antagonist

## Introduction

Chili *(Capsicum annuam)* is one of the most growing crops in the world. Chili fruit is highly infected by *Phytophthora capsici* causing fruit rot. It was first described in 1922 by Leonian at Mexico on chili crop [1]. *Phytophthora capsici,* an oomycete, is a challenging pathogen at chili producing areas of the world. *C. annuam* & *P. capsici* have very complex pathosystem as compare to other *Phytophthora* pathosystems because this pathogen infects all the plant parts [2]. This pathogen causes not only fruit rot but also transmitted by seeds [3]. It has vast host rang and cause diseases also on pumpkin, melons, tomato, eggplant, lima beans and squash. High moisture is an important factor for disease development and pathogen reproduction. Use of synthetic fungicides known as best management strategy for this disease but continuous application of fungicides leads to resistance in pathogens and human health hazards [4]. Large scale adoption of organic farming has boosted up chili production in an eco-friendly way Insensitivity of *P. capsici* against metalaxyl and mefenoxam (Ridomil gold) is reported in Italy and United states [5]. So, there is a need of best alternative management practices that can minimize the disease development with no or less environmental and human health hazards. In this regard, currently usage of biological control agents stands first to suppress the induction of pathogen. *Bacillus* and *Trichoderma* species are extensively used as biological control agent against *Phytophthora* species [6]. Against fungal plant pathogens, *Trichoderma* is known as aggressive biological control agent [7]. It has different complex interaction mechanisms like antibiosis, mycoparasitism and competition to antagonist the plant pathogens [8]. Many species of *Trichoderma* produce volatile and non-volatile metabolites like massoilactone, viridine, furanone, trichodermine, peptaibols, harianic acid,

gliovirin, heptelidic acid which are noxious to pathogens [9]. Use of *Trichoderma* also induces systemic resistance in some plants belonging to Poaceae, Solanaceae and cucurbitaceae family against some plant pathogens [10]. Two species of *Bacillus* reduced the mycelial growth of *P. capsici* [11]. Present study was designed to evaluate the antifungal ability of fungal and bacterial biocontrol agents against fruit rot disease of chili.

## Materials and Methods

Diseased chili fruit samples were collected from chili growing area of district Faisalabad, Punjab, Pakistan. Isolation was performed by using tissue segmented method on PARP medium and identification was done as described by [12]. Three molecular characterized isolates of Trichoderma namely *Trichoderma asperellum* TH, *Trichoderma harzianum* HM, *Trichoderma harzianum* HK were taken from Plant Pathology laboratory, College of Agriculture, University of Sargodha. For *Bacillus* isolation, soil samples from chili field were collected and isolation was done by using serial dilution technique. About 50µl solution was spread on PDA plates and incubated at 25±1°C for 36 hours [13]. Identification was performed based on morphological and cultural characters as described by [14].

### Antagonistic Activity

#### Dual culture

**For *Trichoderma* isolates:** Five days old, 6mm mycelial plug of pathogen and antagonist were placed opposite to each other aseptically on petri plates which contains sterilized PDA medium and sealed with parafilm.

**For *Bacillus* isolates:** Five days old, 6mm mycelial plug of pathogen was placed at one side of sterilized PDA plate, then a loop full of two days old culture of *Bacillus* was streaked out on the opposite side of mycelial plug of pathogen and sealed with parafilm and incubated at 24±1°C. Petri plate containing only pathogen was served as control.

### Metabolites

#### Volatile metabolites

To evaluate the antifungal activity of volatiles metabolites of *Trichoderma* against *Phytophthora capsici*, technique described by [15] was used. Five days old, 6mm mycelial plugs of *Trichoderma* isolates and *P. capsici* were placed at center of PDA petri plates aseptically, plates containing *P. capsici* were placed over on *Trichoderma* containing plates for three days. All petri plates were sealed with parafilm and incubated at 25±1°C. Same procedure was used for volatile metabolites of *Bacillus* isolates.

#### Culture filtrate / Nonvolatile metabolites

**For *Bacillus***

To evaluate the antifungal activity of culture filtrates of two *Bacillus* isolates against *P. capsici*, technique described by (Abdulkareem *et al*., 2014) was used. A 6mm plug of *Bacillus* isolates were inoculated into 100ml nutrient broth agar and shaked at 150rmp for 48 hours at room temperature. The broth culture was filtered by Whatman No.1 filter paper and centrifuged at 6000rmp for 12min. To obtain cell free culture, liquid was re-filtered by Millipore membrane filter (0.2µ). Twenty ml cell free culture was mixed in eighty ml sterilized PDA media by using food poison technique. After the solidification of media, 6mm mycelial plugs of *P. capsici* were placed at center of PDA petri plates aseptically. PDA petri plates without culture filtrate were served as control. All petri plates were sealed with parafilm and incubated at 25±1°C.

**For *Trichoderma***

To evaluate the antifungal activity of Extract of metabolites (culture filtrates) of three *Trichoderma* isolates against *P. capsici*, technique described by [16] was used with minute modification. Five days old, sex mycelial plugs of *Trichoderma* isolates were inoculated in 100ml liquid potato dextrose broth (PDB) and shaked at 200rpm for 70 hours at room temperature. Then broth culture was centrifuged at 8000rpm for 25min. To obtaining cell free culture, broth culture was first filtered by filter paper (Whatman No. 1) and then passed through Millipore membrane (0.34µm). Twenty ml cell free culture was mixed in eighty ml sterilized PDA media by using food poison technique. After the solidification of media, 6mm mycelial plugs of *P. capsici* were placed at center of PDA petri plates aseptically. PDA petri plates mixed with distilled water instead of culture filtrate were served as control. All petri plates were sealed with parafilm and incubated at 25±1°C.

### Statistical analysis

The data was analyzed by using factorial test on Statistics software for the interpretations of results. Difference between means were calculated by using LSD test. Percentage inhibition was determined by using following formula (Vincent, 1927). Inhibition percentage (%) = C-T/C × 100

Where C = growth of pathogen in control plate, and T = mycelial growth of pathogen in test plate.

## Results

### Dual culture

In dual culture technique, treatment ($F_{8, 170.75}$=149.30) effect was significant. *T. asperellum* TH showed highest inhibition (61.65%) among all other tested treatments. *Bacillus* A showed 54.36% inhibition followed by *T. harzianum* HK (51.43%), *T. harzianum* HM (47.26%) and *Bacillus* B (41.56%).

### Metabolites

#### Volatile metabolites

In volatile metabolites, treatment ($F_{4, 121.16}$=207.45) effect was significant. Volatile metabolites of *T. asperellum* TH showed maximum inhibition (28.34%) against tested pathogen followed by *T. harzianum* HK (22.89%), *T. harzianum* HM (19.72%), *Bacillus* A (16.56%) and *Bacillus* B (11.51%).


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**Culture filtrate/ extracted metabolites (Nonvolatile)**

In nonvolatile metabolites treatment ($F_{4, 109.009}$= 445.7) effect was significant. Nonvolatile metabolites of *T. asperellum* TH showed maximum inhibition (44.58%) among all tested other treatments followed by *Bacillus* A (38.75%), *T. harzianum* HK (36.58%), *T. harzianum* HM (31.85%) and *Bacillus* B (29.16%) (Table 1).

**Table1:** Effect of Biological Antagonists on Mycelial Growth of *P. capsica*.

| % Growth Inhibition of Phytophthora *Capsici* | | | |
|---|---|---|---|
| **Antagonist** | **Dual culture** | **Volatile metabolites** | **Nonvolatile metabolites** |
| *T. harzianum* HM | 47.26 c | 19.72 c | 31.85 d |
| *T. harzianum* HK | 51.43 b | 22.89 b | 36.58 c |
| *T. asperellum* TH | 61.65 a | 28.34 a | 44.58 a |
| *B. subtilus* A | 54.36 b | 16.56 d | 38.75 b |
| *B. subtilus* B | 41.56 d | 11.51 e | 29.16 e |

## Discussion

Management of phytopathogens through antagonistic biocontrol agents is more efficient, long lasting and nonchemical approach that is widely adopted in the world. Present study assessed the antifungal ability of different biocontrol agents by dual culture, volatile and nonvolatile metabolites. *T. asperellum* TH showed highest inhibition among all other tested antagonist in different assays. *Bacillus* isolates gave less inhibition percentage of radial growth of tested pathogen as compared to *Trichoderma* isolates. This may be due to microbial interaction like compatibility, antibiosis and stimulation levels of *Trichoderma* and *Bacillus* against *P. capsici* [17,18]. Antifungal activity of *Bacillus* against soil born phytopathogens was also reported [19]. Previously similar results about the antagonism of *Trichoderma* isolates against *P. capsici* was reported [20]. Lelay *et al.* (2007) reported the antifungal ability of six *Trichoderma* isolates that inhibited the mycelial growth of *Rosellinia necatrix* about 14 to 27%, it was accredited to the production of metabolites like viridine, glyotoxins, furanone, trichodermine, and 6-pentyl-α-pyrone. In present study, *Bacillus* isolates showed the inhibitory effect against tested pathogen as compared to control treatment [21]. Same isolates of *Trichoderma* and *Bacilllus* species which were used in present study, inhibited the growth of *Phytophthora drechsleri* under laboratory and field conditions [15], so our results also confirmed the antifungal ability of these biocontrol agents against *P. capsici*.

## Conclusion

According to present study it is concluded *Bacillus* and *Trichoderma* isolates have great potential to manage the growth of phytopathogens. We can use them to obtain organic crops and manage the plant diseases safely as compared to synthetic chemicals that are harmful to human health and environment. Chemicals gave short duration control of any plant disease while bio control agents gave long lasting control of diseases. It is concluded that *Trichoderma* and *Bacillus* isolates and their metabolites have great potential to manage the *Phytophthora capsici* causal agent of fruit rot of chili with less or no residual effects.

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