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In vitro Evaluation of the Antagonistic Activity of *Trichoderma* sp. against *Fusarium verticillioides*

Ann Jhudeil C. Santos[1*], Cynthia C. Divina[1], Federico G. Pineda[1], and Lani Lou Mar A. Lopez[2]

[1]Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University; [2]Ramon Magsaysay Center for Agricultural Resources and Environment Studies, Central Luzon State University, Philippines.

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Fusarium verticillioides is an agriculturally important fungus widely distributed throughout the world, infecting a wide range of plant hosts in all stages of development. This study determined the antagonistic effects of *Trichoderma* sp. against *F. verticillioides* under *in vitro* conditions. Mycelial growth inhibition of *F. verticillioides* by *Trichoderma* was determined using bi-culture test. The microscopic interactions between the two fungi were studied using compound light microscope. Results of bi-culture test indicated that *Trichoderma* can inhibit the mycelia growth of *F. verticillioides* having an average inhibition growth rate of 32% over control. Drying up of the pathogen from the point of contact with the antagonist was also observed in the bi-culture experimental plates. This phenomenon is an indication that the pathogen was depleted of nutrients in presence of the antagonist. Using slide-biculture technique, it was revealed that *Trichoderm* sp. can cause damages to *F. verticillioides* including cell lysis and disintegration of the hyphae. Entry of the hyphae of *Trichoderma* sp. into the lumen of *F. verticillioides* and parasitic behavior by coiling round to the hyphae of the pathogen were also observed under compound light microscope. These results suggest that *Trichoderma* sp. uses antibiosis, mycoparasitism, and competition for space and nutrients as suppression mechanisms against *F. verticillioides in vitro.* Therefore, *Trichoderma* sp. has the potential to be a bio-control agent of *F. verticillioides* in certain crops. However, further studies on the effectiveness of *Trichoderma* sp. to control *F. verticillioides* should be conducted under *in vivo* conditions.

Keywords: *Trichoderma* sp., *Fusarium verticillioides*, biocontrol agent, *in-vitro, in-vivo*

[*] **Corresponding author:** Ann Jhudeil C. Santos; **E-mail:** annjhudeilsantos@gmail.com

Introduction

Fusarium verticillioides is an important multi-phytopathogenic fungus with a wide range of hosts such as maize, sorghum, rice, and millet. This fungus can infect plants in all stages of development, from the early hours of seed germination to the time of harvest, including post-harvest deterioration of grains, resulting in huge economic losses (Deepa and Sreenvasa, 2017). Among the *Fusarium* species, *F. verticillioides* is the most prominent producing a wide range of mycotoxins that includes fusaric acid, fusarins, and fumonisins (Desjardin *et al*., 2010). Fumonisins are the most widespread of these mycotoxins (Munkvold, 2003) and it can be toxic for both humans and animals (Duan *et al*., 2016).

For controlling different diseases such as *F. verticillioides*, farmers usually apply synthetic fungicides to the plants. However, these treatments are useless because of toxic and harmful effects to the environment and health. The disturbing issues regarding the noxious effects of these chemical pesticides lead into an increased demand for products coming from sustainable agriculture and precision farming. Precision farming enables farmers to maximize crop production while utilizing minimum farm inputs especially inorganic chemicals such as pesticides and fertilizers (Dar and Soytong, 2014). Biological controls in recent times have been accepted as more natural and environmentally-acceptable alternative to the existing chemical treatments. It is one of the most promising tools to maintain current level of agricultural production (Mishra *et al*., 2011). Nowadays, many microorganisms have been used as biological control agent of plant diseases and one of these is *Trichoderma* sp. *Trichoderma* is known worldwide as a fungus with high antagonistic properties against fungal pathogens. These properties were established in several laboratories and field experiments conducted in the Philippines and other countries against a wide range of soil-borne and airborne plant pathogens of various crops (Cuevas *et al*., 2005). In addition to disease control, *Trichoderma* treatment promoted growth of the crops that was translated into significantly higher agronomic yield.

Since increased incidences of plant diseases caused by *Fusarium verticilloides* have become an alarming issue and the use of fungicides caused negative effects to environment and health, serious attention must be paid to find an alternative way to control this fungal pathogen.

Objectives: The purpose of this study was to evaluate the antagonistic effects of *Trichoderma* sp. against *F. verticillioides* under *in vitro* conditions.

Materials and methods

Source of Trichoderma sp. and Fusarium verticillioides

Pure culture of *Trichoderma* sp. was acquired from the CLSU Ramon Magsaysay Center for Agricultural Resources and Environment Studies (RM-CARES) and the pure culture of *Fusarium verticillioides* was obtained from the Philippine Center for Post Harvest Development and Mechanization (PhilMech).

B-iculture of Trichoderma sp. and F. verticillioides

A 6 mm cork borer was used to make agar blocks of *Trichoderma* sp. and *F. verticillioides*. The agar blocks of both fungi were placed with a distance of approximately 6 cm opposite to each other on petri plates containing sterilized PDA. The plates were run in five replicates with two control set; one containing only pathogen and the other one with the antagonist only. The Petri plates were incubated at room temperature. The diameter of the growth of mycelia of *F. verticillioides* and *Trichoderma* sp. isolates were measured using digital vernier caliper. The data were recorded every 24 hours.

Percentage of mycelia growth inhibition was calculated according to the formula:

$$MGI\% = (dc - dt) \times 100/dc$$

Where:
dc= colony diameter of the pathogen in control sets
dt= colony diameter of the pathogen in treatment sets

Slide Bi-culture

Approximately 6mm agar block of *F. verticillioides* was grown at the center of the glass slide containing thin film of sterilized PDA. *Trichoderma* sp. was grown with a distance of approximately 3 cm away from the pathogen. The slides were then placed into a sterilized petri dish in an elevated manner. The petri dishes were lined with moist sterile filter paper which serves as a moist chamber. It was periodically observed for almost one week under the compound light microscope.

Slide bi-culture was done to document the possible biological relationship between *Trichoderma* sp. and *F. verticillioides*.

Results and Discussion

Antagonistic Activities of Trichoderma sp. Against Fusarium verticillioides Using Biculture Technique

Trichoderma sp. was evaluated for its antagonistic ability against *F. verticillioides* by preparing bi-culture plates. It was observed that after the inoculation of both fungi in PDA plates, antagonist grew faster compared to the pathogen. By day 3 of incubation, the antagonist occupied almost half of the plate in all replicates, leaving very little space for the growth of pathogen. At the 7th day of incubation, *Trichoderma* sp. covered almost ¾ of the plate. The comparison of the means revealed that the mycelial growth of *Trichoderma* sp. was significantly bigger compared to *F. verticillioides* having an average growth of 6.53 cm and 2.77 cm, respectively. After 14 days, the percentage of mycelial growth inhibition was calculated as shown in Figure 1. Results indicated that *Trichoderma* sp. inhibited the mycelia growth of *F. verticillioides* to an extent of 23.86% to 43.14% over control but with mean of 32%.

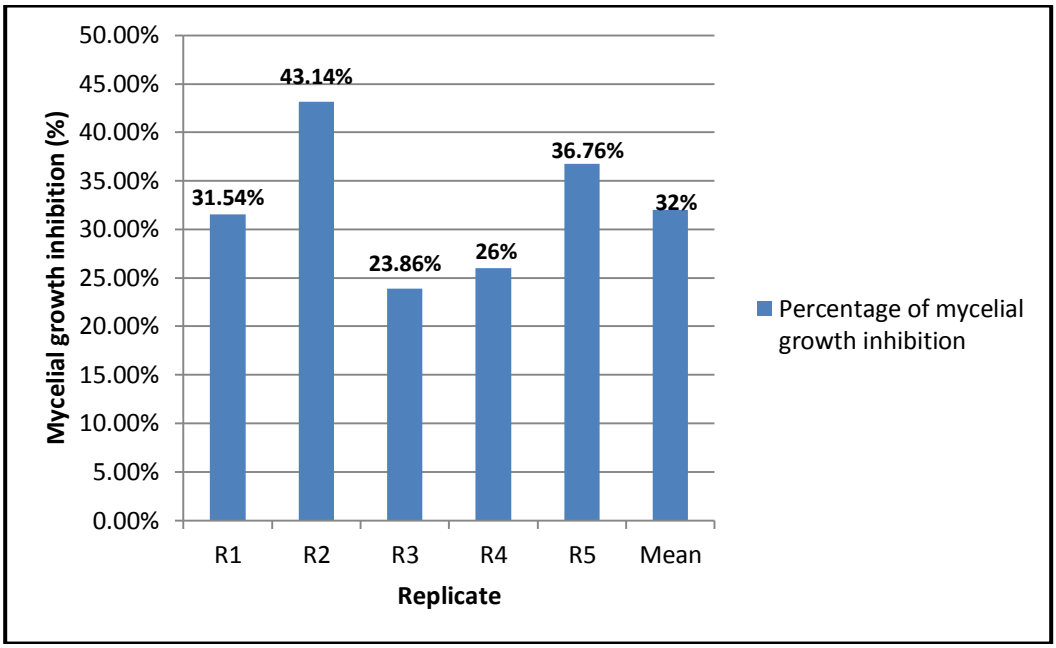

Figure 1. Percentage of mycelia growth inhibition of *F. verticillioides* by *Trichoderma* sp. after 14 days of incubation period.

Figure 2 illustrates the interaction of *Trichoderma* sp. and *F. verticillioides*. It shows that *Trichoderma* sp. ramified the plate faster, therefore, inhibiting the further growth of the pathogen. No clear contact between the antagonist and pathogen was observed up to 15th day of incubation. *Trichoderma* sp. showed no signs of mycelial colonization, but somewhat produced mycelial inhibition to *F. verticillioides*, this is due to the presence of a clear zone between both fungi. Drying up of the pathogen from the point of contact with the antagonist was also observed on replicates, a phenomenon which was not observed on the pure culture of the pathogen (Fig.2d). Fig. 3b shows that *F. verticillioides* was obviously depleted of nutrients. This occurrence was also observed by Sobowale *et al*. (2009) in his study on the interaction of *T. harzianum* strains and *F. verticillioides*.

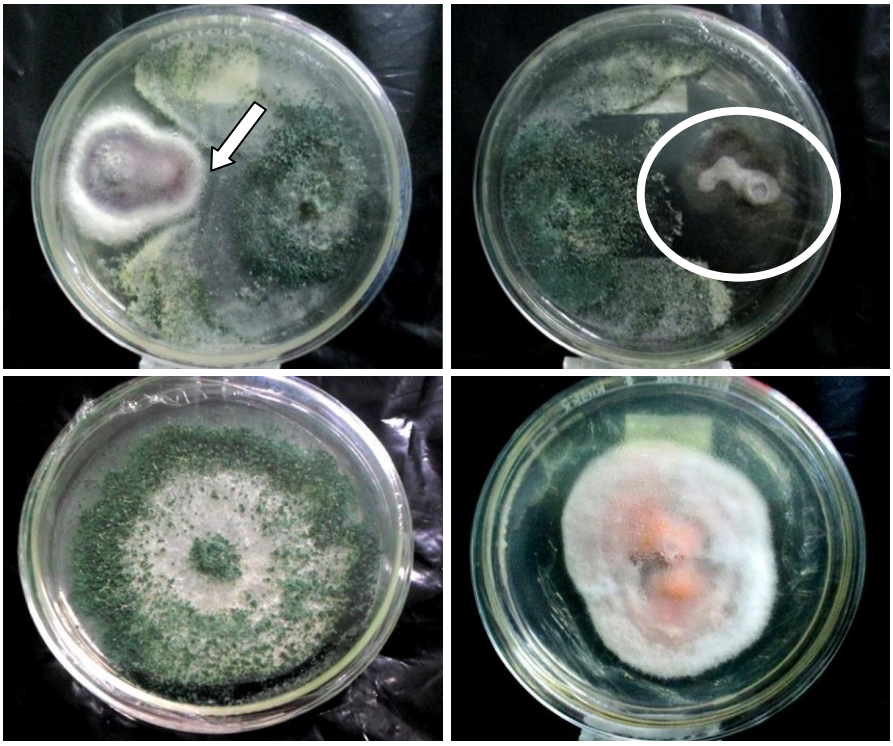

Figure 2. Bi-culture plates of *Trichoderma* sp. and *F. verticillioides* at 15 days of incubation. Clear zone formed between *Trichoderma* sp. and *F. verticillioides* (a). Drying up of the mycelial mass of *F. verticillioides* (b). Control plate of *Trichoderma* sp. (c). Control plate of *F. verticillioides* (d).

According to Kumar *et al*. (2012), the rapid growth of *Trichoderma* isolates added an advantage to inhibit the growth of pathogens by competing for space and nutrients even before it deploys mycotoxins. In addition, the drying of the pathogen from the point of contact with the antagonist is also a sign of competition for nutrients and space as described by Deacon and Berry (1992). The heavy sporulation observed in petri plates is an evidence of mycoparasitism which according to Sobowale *et al*. (2009) is one of the suppression mechanisms of *Trichoderma* sp.

The clear zone which was observed suggest a possible production of colorless metabolites by the antagonist which moved ahead of them, occupying space within the agar, therefore inhibiting further growth of *F. verticillioides*. The result of the present study clearly signified that *Trichoderma* sp. is a promising antagonistic fungus against *F. verticillioides*.

Microscopic Observation of Trichoderma sp. and Fusarium verticillioides Interaction on Slide Culture

Trichoderma sp. and *F. verticillioides* were observed under the microscope to determine the different microscopic interactions between the two fungi. Figure 3 shows the growth of *Trichoderma* sp. and *F. verticillioides* on slide culture. There were no physical interactions between the antagonist and pathogen observed. The presence of clear zone between the two fungi evidently confirms that *Trichoderma* sp. exerts inhibitory effect on *F. verticillioides*. This occurrence supported the results obtained in bi-culture test presented.

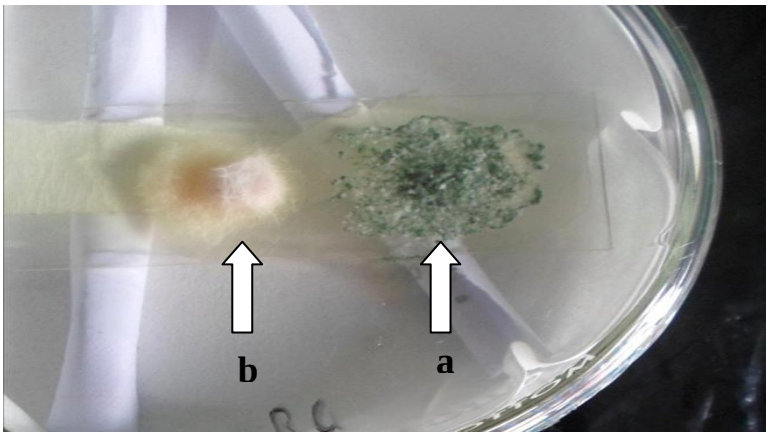

Figure 3. *Trichoderma* sp. **(a)** and *F. verticillioides* **(b)** on slide culture at 3 days.

Figures 4 and 5 show the photomicrographs of *Trichoderma* sp. and *F. verticillioides* under compound microscope. As observed under the microscope, the hyphae of *F. verticillioides* showed abnormal morphology such as the occurrence of bead-chain appearance (Fig. 5c), that is due to the entry of the hyphae of *Trichoderma* sp. into the lumen of *F. verticillioides*. Cell lysis (Fig. 5d) and hyphal disintegration (Fig. 5b) were also observed in the mycelia of *F. verticillioides* when grown with the antagonist, an observation which was different compared to the mycelia of *F. verticillioides* which was free from *Trichoderma* sp. The hyphae of *F. verticillioides* grown away from *Trichoderma* sp. showed normal morphology (Fig. 5a). These observations concurred with the results obtained by Sharma (2011) in his study about the *Trichoderma-Fusarium* interaction.

Trichoderma sp. also showed parasitic behavior to *F. verticillioides* by attaching itself to the pathogen, coiling round and strangulating the hyphae (Fig. 5f.). Similar interactions were observed by Nayaka *et al*. (2008). The study of Howell (2003) demonstrated the ability of *Trichoderma* to grow tropically toward the hyphae of other fungi, coil around them in a lectin-mediated reaction, and degrade the cell walls of the target fungi by the secretion of different lytic enzymes. This process (mycoparasitism) limits the growth and activity of plant pathogenic fungi.

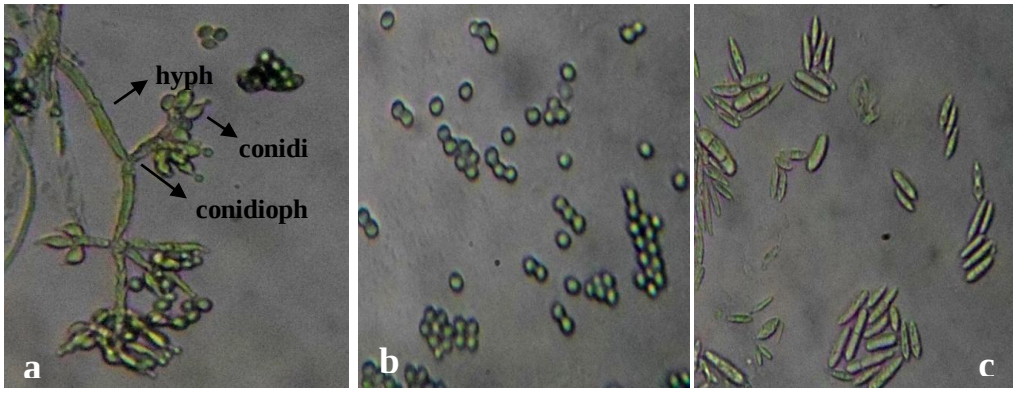

Figure 4. The microscopic morphology of *Trichoderma* sp. and *F. verticillioides* under compound microscope at 40x magnification. Morphological features of *Trichoderma* sp. (a). Spores of *Trichoderma* sp. (b). Conidia of *F. verticillioides* (c).

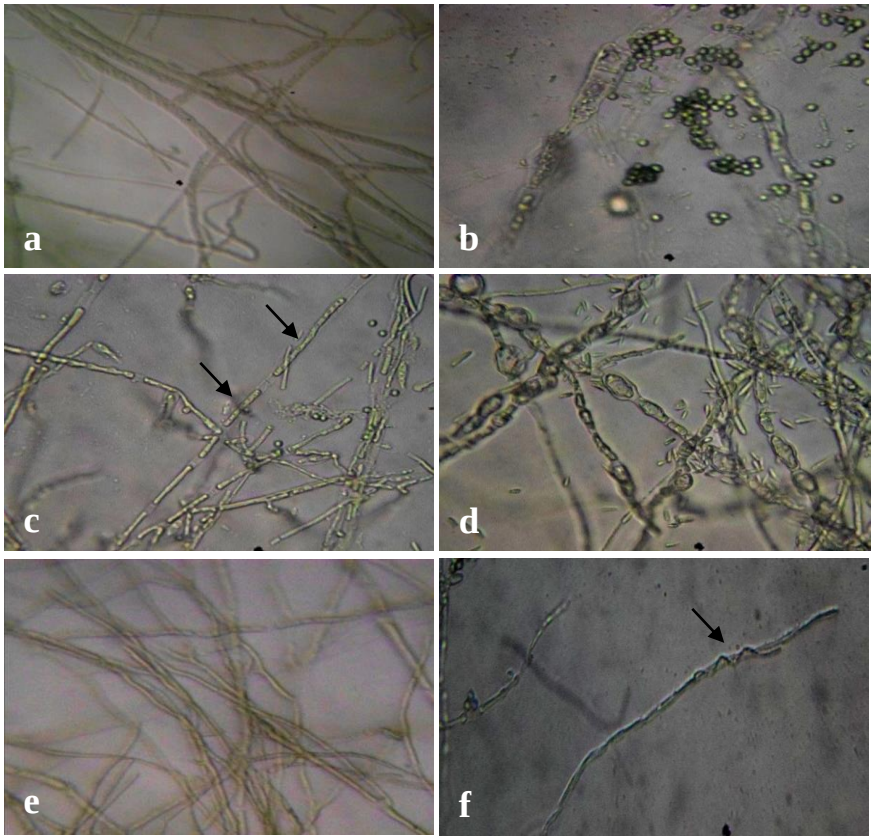

Figure 5. Normal hyphae of *F. verticillioides* under binocular compound microscope at 40x magnification (a). Disintegration of the hypha of *F. verticillioides* (b). Entry of the hyphae of *Trichoderma* sp. into the lumen of *F. verticillioides* (c). Cell lysis of *F. verticillioides* (d). Normal hyphae of *Trichoderma* sp. (e). Coiling of the hypha of *Trichoderma* sp. over *F. verticillioides* (f).

Based on the results, it could be inferred that *Trichoderma* sp. may produce compounds that could cause damages to the hyphae of *F. verticillioides*. According to Benitez *et al.* (2004), antibiosis gives *Trichoderma* the ability to attack fungal pathogens by the production of toxins or enzymes that cause the hyphae of the pathogen to shrivel, disintegrate, or even lead to death.

Conclusion

Trichoderma sp. uses antibiosis, mycoparasitism, and competition for space and nutrients as suppression mechanisms against *F. verticillioides in vitro*. Antibiosis of *Trichoderma* sp. can cause damages to the hyphae of *F. verticillioides* and mycoparasitism can cause coiling of the hyphae of *Trichoderma* around the pathogen. Therefore, *Trichoderma* sp. has the potential to be a bio-control agent of *F. verticillioides* in certain crops. However, further studies should be conducted under *in vivo* conditions to evaluate the effectiveness of *Trichoderma* sp. against *F. verticillioides* under the presence of biotic and abiotic conditions.

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