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Antagonistic bio-control potentiality of *Trichoderma harzianum* in controlling the growth of *Fusarium solani* and *Sclerotium rolfsii* under *in-vitro* conditions

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Abstract
In this study, antagonistic bio-control potentiality of *Trichoderma harzianum* on the growth damping-off pathogens *Fusarium solani* and *Sclerotium rolfsii* was evaluated under *in- vitro* condition on Potato Dextrose Agar (PDA) medium. Results showed the growth inhibition of *Fusarium solani* and *Sclerotium rolfsii* to the extent of 77.8% and 66.7%, respectively in comparison with solo culture plate of test pathogen which exhibited 0% inhibition. Thus *Trichoderma harzianum* can be a best tool for the biological control of test pathogens.

Keywords: Bio-control, damping-off, inhibition and antagonistic

Introduction

Damping-off is a disease condition caused by *Rhizoctonia* spp., *Pythium* spp., *Fusarium* spp., *Sclerotium* spp. Altogether; damping-off is caused by a number of biotic or abiotic factors, which prevent seeds to germinate and seedlings to emerge from soil. Damping-off pathogens affects seeds and seedlings of almost all plants including field, horticulture and plantation crops (Kraft *et al*. 2000) [9].

Fusarium solani (Current name: *Neocosmospora solani* (Mart.) L. Lombard and Crous) is a soilborne fungal plant pathogen found in agricultural soils marking a worldwide distribution. It infects several plant species of diversified plant families. Chief host plants among them are potato, tomato, chilli, onion, brinjol, bean, pea and all the members of cucurbitacea family such as water melon, musk melon, cucumber, bottle guard, ridge guard and squash guard. *Fusarium solani* induces plants to undergo damping-off, root rot, stem rot, surface rot and corn rot. *F. solani* produces scarce to plentiful white to cream colored mycelium on potato dextrose agar (PDA) medium. Colonies of *F. solani* are fast growing, develops variable color and texture, sometimes granular to fluffy, rose-red color, purple, or lavender color. Colony starts out as white, cottony growth that gradually turns dark with maturity (Zaccardelli *et al*., 2008) [12].

Sclerotium rolfsii Sacc (Current name: *Athalia rolfsii* (Curzi) C.C. Tu and Kimbr.) is a soil borne fungal plant pathogen exhibiting diverse host range and ubiquitous in soils worldwide, able to cause root rot and damping-off disease in tomato, cucurbits, potato, pepper, coriander, cantaloupe, celery, okra, carrot, cauliflower, cabbage, bean, eggplant, and groundnut, including several crops having economic importance (Christias, 1980) [5].

Conditions that enhance the severity of damping-off includes higher soil moisture regime in the root zone, excessive overhead irrigation, lower soil temperatures before emergence, higher soil temperatures after seedling emergence, and plant overcrowding or thick plant population. These pathogens may spread via, irrigation water run-off or rain, mono-cropping, working in the contaminated soil, improperly sanitized tools, introduction of infected plants, improperly sanitized greenhouse, and the use of contaminated water for irrigation water (James 2012; Starkey and Enebak 2012) [8, 11].

Once established, it is difficult to manage damping-off pathogens as they are recurring season by season are able to survive for many years in the soil through there resting spores and specialized structure. Even in the absence of host plants, pathogen survives as saprophytes and is capable of overcoming adverse conditions (Agrios, 2005) [2].

Economic impact from damping- off can be evaluated as direct cost, due to damages of seed germination or seedlings and an indirect cost, as an additional cost of replanting and yield loss (Babadoost and Islam 2003; Bacharis *et al*. 2010; Horst 2013) [3, 4, 7]. Till now, there is no detailed and accurate estimation about the actual economic impact of damping-off at the global level in monetary terms. As per the available research works incidence of damping-off may vary from 5 to 80%.

Controlling soil borne disease in general and damping-off disease in particular is often a difficult task. Chemical fungicides give an excellent control, but have far reaching consequences on environment and ecological entities. Exploiting naturally occurring biological control agent for managing plant diseases is a safe and eco-friendly approach. *Trichoderma harzianum* is a bio- control agent known to inhibit the pathogens antagonistically by the way of competing with the soil pathogen through its fast-vigorous growth and fast exploitation of nutrients from media.

In this present study, efforts have been directed to know the antagonistic bio-control properties of *Trichoderma harzianum* on the growth of *Fusarium solani* and S*clerotium rolfsii*.

Materials and Methods

Sample collection, isolation of pathogen fungi and their identification

Studies were carried out at the Department of Botany, Faculty of Science, B. N University, Udaipur district of Rajasthan. Damping-off diseases affected onion seedling samples were randomly collected from nursery and the adjoining field from Udaipur region. The diseased plants were collected in the polythene bags and were transported to the laboratory for the purpose of isolating damping-off pathogens from the infected root bits. Infected root bits of samples were gently washed under tap water for about a minute to remove any dirt and soil particles. The root pieces (0.5 cm) were dipped in 0.01% $HgCl_2$ for about 15 seconds and then passed from three washes of distilled sterile water for 2-3 minutes each to remove the traces of $HgCl_2$. The treated root pieces were dried completely in the aseptic condition and then transferred to Petri plates containing sterilized potato-dextrose agar (PDA) medium at the rate of 5-6 pieces/ Petri plate. All the Petri plates were kept at 25 ± 2 ºC for 7 days. The colonies which were showing distinct mycelial growth habit were segregated by hyphal tips and transferred on to the fresh potato dextrose agar (PDA) medium. The purified fungus cultures were maintained on PDA slants in test tubes for further studies. The growth is sub-cultured/multiplied whenever needed during the entire study. Isolated fungal pathogens were sent to Agharkar Research Institute, Pune for the purpose of pathogen identification.

Trichoderma harzianum and pathogen dual culture

Mycelial bits (5mm) of freshly grown 7 days old cultures of *Trichoderma harzianum* (Plate. 1) and a single fungal pathogen each time were placed on the PDA plates at 4 cm apart. The plates were incubated at 25 ± 2 ºC for 7 days. Control plates contain only a single pathogen without *Trichoderma harzianum*. Antagonistic effect of *Trichoderma harzianum* against fungal pathogens were measured using following formula (Kucuk and Kivance, 2004) [10].

% MGI = DC-DT/DC x 100

where: % MGI = percentage of mycelium growth inhibition

DC = diameter of the fungal colony in control and

DT = diameter of the fungal colony in treatment

Results and discussion

Fungi were identified by Agharkar Research Institute, Pune as *Fusarium solani* (Mart.) Sacc. (Current name: *Neocosmospora solani* (Mart.) L. Lombard and Crous) and *Sclerotium rolfsii* Sacc (Current name: *Athalia rolfsii* (Curzi) C.C. Tu and Kimbr.). These test fungal pathogens were separately tested on dual culture involving *Trichoderma harzianum*. *Trichoderma harzianum* exhibited antagonistic biological control activity against two of the fungal pathogens. Results showed the growth inhibition of *Fusarium solani* and *Sclerotium rolfsii* to the extent of 77.8% and 66.7%, respectively in comparison with solo culture plate of test pathogen which exhibited 0% inhibition (Plate 2 and 3). Similar antagonistic effect of *Trichoderma* spp. on *Macrophomina phaseolina* was reported. *Trichoderma harzianum* biologically controls several soil borne diseases including *Rhizoctonia solani, Rhizoctonia bataticola* and *Exserohilum turcicum*, through its fast vigorous growth and fast exploitation of nutrients from media (Adekunle *et al*., 2001; Harlapur *et al*., 2007) [1, 6].

Conclusion

Trichoderma harzianum can be a better alternative for chemical fungicides for controlling soil borne disease, including damping-off caused by *Fusarium solani* and *Sclerotium rolfsii*. *Trichoderma harzianum* can be a perfect tool in integrated disease management programme.

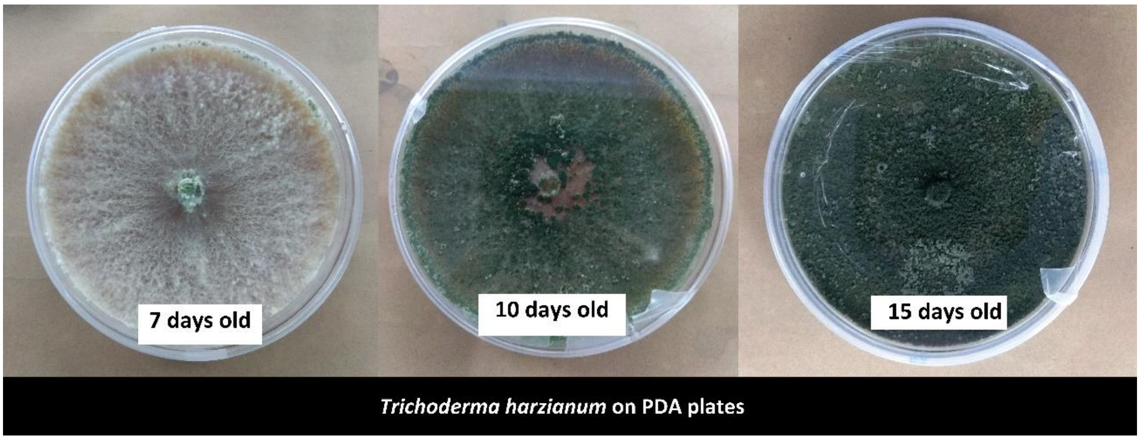

Plate 1: Color development in *Trichoderma harzianum*

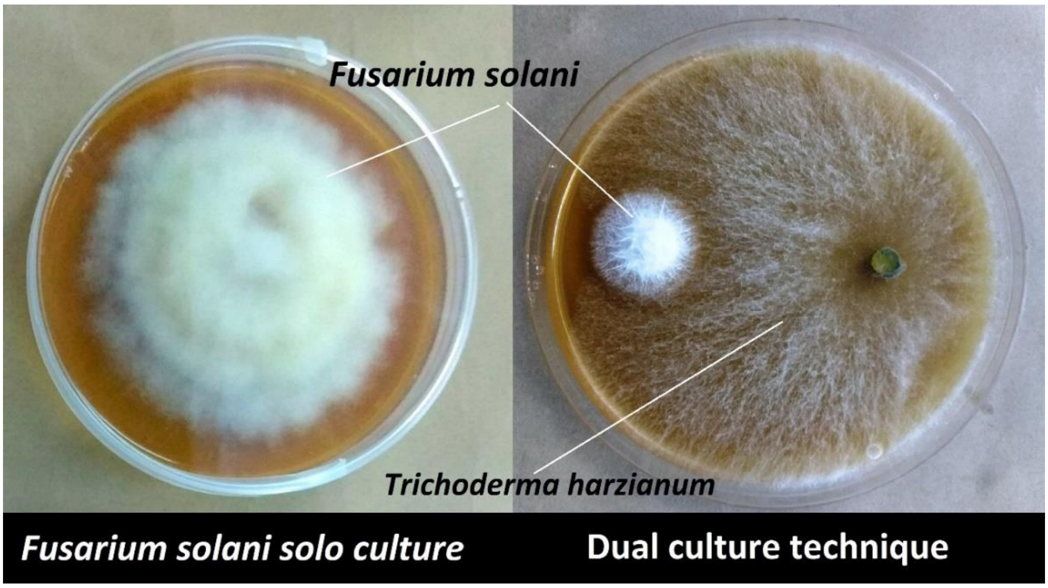

Plate 2: Dual culture of *Fusarium solani* and *Trichoderma harzianum*

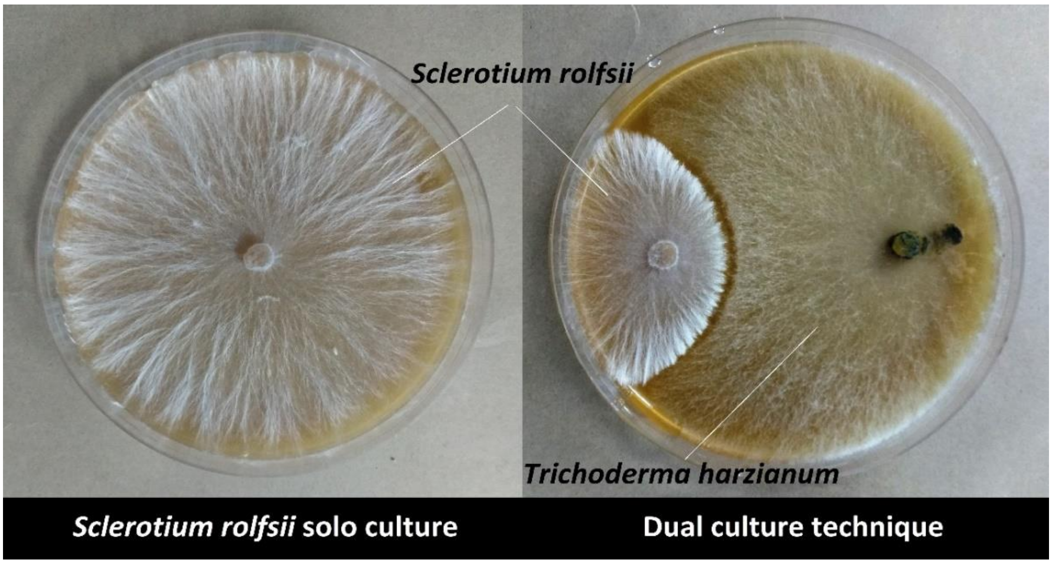

Plate 3: Dual culture of *Sclerotium rolfsii* and *Trichoderma harzianum*

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