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In Vitro Antagonistic Activity of *Trichoderma* and *Penicillium* species against *Macrophomina phaseolina* (Tassi) Goid

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ABSTRACT

A study was undertaken to evaluate the antagonistic activity of seven Trichoderma species, and two Penicillium species against brinjal root rot causing pathogen, Macrophomina phaseolina (Tassi) Goid by dual culture plate technique under in vitro conditions. All the biocontrol agents showed considerable reduction in the growth of the pathogen. Among the seven Trichoderma species studied, Trichoderma harzianum showed maximum antagonistic activity of 77.77% followed by T. pseudokoningi 74.44%, T. koningi 72.22%, T. virens, T. viride, T. reesei 70% each, T. atrovireide 66.66%, Penicillium islandicum 57.77% and P. aurantiogriseum 55.55%. The results of the present study suggest that T. harzianum has a highly antagonistic potential against the test pathogen.

Key words: Biocontrol, brinjal, inhibition, rhizosphere.

INTRODUCTION

Pesticide usage in agriculture leads to environmental pollution, causing hazardous effects to both environment and food quality. Many pathogenic microorganisms have developed resistance against chemical fungicides [1]. Fungicides pose serious hazards to health and environment. This emphasized an alternative method to control fungal diseases. Biocontrol of plant pathogen is an ecofriendly, safe approach that utilizes antagonistic microorganisms as a potential means of disease control. *Trichoderma* is a non-pathogenic biocontrol agent having antagonistic properties against many plant pathogens in various degrees [2, 3]. Genus *Penicillium* produces both antibacterial [4, 5] and antifungal compounds [6]. *Penicillium* spp. was used as a root colonising fungi to control Fusarium wilt of tomato [7].

To determine the antagonistic property of *Trichoderma* spp. and *Penicillium* spp. against *Macrophomina phaseolina* (Tassi) Goid (Mp), isolates were compared on a medium and at temperature where both antagonist and Mp can grow well in the laboratory. The present study was undertaken, to find out the biocontrol efficacy of *Trichoderma* spp. and *Penicillium* spp. against Mp.

MATERIALS AND METHODS

The rhizosphere soil samples were collected from brinjal cultivated agricultural fields and the mycoflora were isolated by serial dilution plate technique [8, 9]. Pathogenic fungi, Mp was isolated from the diseased parts of brinjal during field survey in Kodad, Suryapeta, Khammam, and Ibrahimpatnam of Telengana State, India. *Trichoderma viride*, *Trichoderma harzianum*, were isolated on modified Trichoderma Selective Medium (TSM), [10, 11]. They were purified by single spore isolation method and maintained on Potato Dextrose Agar (PDA) slants at 4^{o}C in the refrigerator. *T. virens, T. atroviride, T. koningi, T. pseudokoningi, T. reesei*, were procured from Mycology and Plant Pathology Laboratory, Department of Botany, Osmania University, Hyderabad. Telangana State.

Antagonist fungi

Trichoderma species were purified by single spore isolation technique, and maintained on PDA slants, and stored in the refrigerator for further use. The *Penicillium* spp. was isolated by soil dilution plate technique on Malt Extract Agar (MEA) medium. *Penicillium* spp. were maintained on MEA slants and stored in the refrigerator.

Dual culture plate technique

Trichoderma spp. and *Penicillium* spp. were evaluated against Mp by the dual culture plate technique [2, 3]. The antagonistic efficacy against test pathogen was evaluated on PDA medium. Both pathogen and antagonists were grown on PDA plates separately for 5 days. Mycelial discs of 6 mm in diameter of antagonist was excised from the edge of an actively growing culture plate and inoculated opposite to the pathogenic fungi in the same plate 1cm away from the edge similarly. For each treatment three replicates were maintained and incubated at 27 ± 2^0 C. Control plates were maintained for test pathogen in triplicate. Both antagonist and test pathogen were placed equidistant from the periphery so that they would get equal opportunity for their growth (Plate 1). After the incubation period, the radial growth of Mp in control, as well as in treatment plate was measured and the percent inhibition was calculated using the formula [12].

Where L = Percentage inhibition of radial growth of pathogen (%)
C = Radial growth of the pathogen (mm) in control
T = Radial growth of the pathogen (mm) in treatment

In dual cultures, *Trichoderma* spp. and *Penicillium* spp. were categorized as effective, based on their ability to over grow and inhibit the growth of the pathogens by giving them a score as per modified Bell's scale [13]. Where R1 = 100% over growth, R2 = 75% over growth, R3 = 50% over growth, R4 = locked at the point of contact.

The mycelial mats from zone of interaction in dual culture plate between pathogen and antagonist were placed on glass slide. The glass slides were stained with lacto phenol cotton blue (HiMedia) to improve the visibility of the hyphae and then observed under a light microscope (CH20i Olympus, India). The hyphal interaction between the mycelia of opposite colonies was studied.

RESULTS AND DISCUSSION

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

Isolates of *Trichoderma* spp. and *Penicillium* spp. were evaluated for their antifungal activity against *M. phaseolina*. Of these antagonist species *Trichoderma* spp. showed significant reduction in terms of radial diameter after the treatment, in comparison with the control. Out of the 9 fungal antagonists studied for their efficacy, *T. harzianum* showed maximum extent of inhibition 77.77%, followed by *T. pseudokoningi* 74.44%, *T. koningi* 72.22%, *T. virens*, *T. reesei* and *T. viride* 70% each, *Penicillium islandicum* 57. 77% and least by *P. aurantiogriseum* 55.55% (Table 1). Observations on the growth and colonization of the test pathogens in dual culture screening by the antagonistic isolates proved that different species of *Trichoderm*a have variation in their ability to inhibit the growth of the pathogen Mp. Among the seven *Trichoderma* spp. tested for their antagonistic activity against the test pathogen Mp, six spp. were observed to lock at the point of contact and were rated as R4 according to Bell's ranking (Table 2). However *T. harzianum* showed maximum zone of inhibition 2 mm compared to others (Plate 1).

The fast growing antagonists caused more growth inhibition of the pathogens may be due to mycoparasitism and competition for space and nutrients. The variation in hyper parasitic potential of different isolates of *Trichoderma* against soil borne fungal pathogens has been reported [13, 15, 16, 17] and the species of *Trichoderma* were effectively selective against pathogenic fungi [13, 17]. *Trichoderma* and *Penicillium* spp. are capable of producing extra cellular lytic enzymes that are responsible for their antagonistic activity [18]. The genus *Penicillium* were reported to produce both antibacterial [4, 5] and antifungal compounds [6].

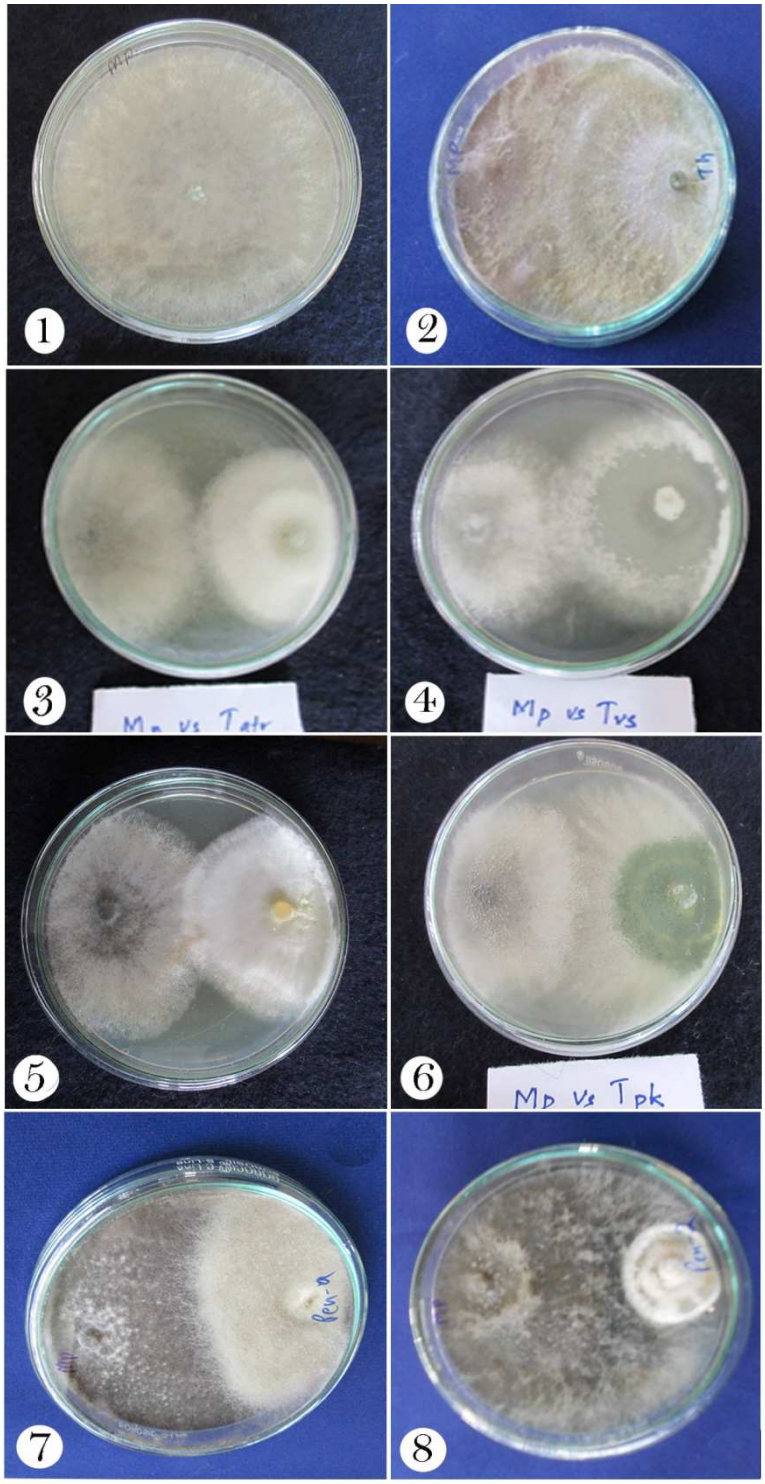

Plate 1. Antagonistic efficacy of different species of *Trichoderma* and *Penicillium* against Mp in Dual Culture Plate method. Figs 1- Mp in control; 2- *Mp vs. T. harzianum;* 3- Mp vs *T. atroviride*; 4- Mp vs *T. virens*; 5- Mp vs *T. koningi*; 6- Mp vs *T. pseudokoningi*; Mp vs *Penicillium isladicum*; 8- Mp vs *Penicillium aurantiogriseum*

Harman et al., (1980) had suggested that mycoparasitism was the principle mechanism involved in controlling Pythium damping-off of pea seed. Hyphal interaction of *Pythium* spp. by *Trichoderma* was also observed *in vitro* by many workers [19, 20]. *Trichoderma* species proved to be superior on account of their faster growth attained against Mp. *Penicillium islandicum* showed slower growth rate and poor competitive ability of these isolates in dual culture which is an indication of their poor antagonistic potential. The variation in hyper parasitic potential of different pathogenic isolates of *Trichoderma* against soil borne fungal pathogens has been reported [15, 16, 17] and the species of *Trichoderma* were differently selective against different pathogenic fungi [2, 3, 21]. This phenomenon may probably be correlated with the differences in levels of hydrolytic enzymes produced by each species or isolates

when they attach the mycelium of the pathogens. *Trichoderma* spp. are capable of producing extracellular lytic enzymes that are responsible for their antagonistic activity [18].

Table 1. Effect of non-volatile compounds of biocontrol agents against *Macrophomina phaseolina*

S. No.	Biocontrol Agent	Radial Growth of the pathogen (mm)	Inhibition (%)
1	Control	90	-
2	*Trichoderma virens*	27	70.00
3	*Trichoderma atroviride*	30	66.66
4	*Trichoderma koningi*	25	72.22
5	*Trichoderma pseudokoningi*	23	74.44
6	*Trichoderma reesei*	27	70.00
7	*Trichoderma harzianum*	20	77.77
8	*Trichoderma viride*	27	70.00
9	*Penicillium aurantiogriseum*	40	55.55
10	*Penicillium islandicum*	38	57.77

Table 2. *In vitro* antagonism of biocontrol agents against *Macrophomina phaseolina*

S. No.	Biocontrol Agent	Time Taken to contact (Days)	Time Taken to Overlap (Days)	Bell's Ranking
1	*Trichoderma virens*	1	Lkd	R4
2	*Trichoderma atroviride*	1	Lkd	R4
3	*Trichoderma koningi*	1	Lkd	R4
4	*Trichoderma pseudokoningi*	1	Lkd	R4
5	*Trichoderma reesei*	1	Lkd	R4
6	*Trichoderma harzianum*	NC	zone of inhibition 2 mm.	-
7	*Trichoderma viride*	1	zone of inhibition 1 mm.	R4
8	*Penicillium aurantiogriseum*	2	zone of inhibition 1 mm.	R4
9	*Penicillium islandicum*	NC	Lkd	R4

NC- no contact, Lkd- locked, R1- complete over growth, R2- 75 % over growth, R3- 50% over growth, R4- locked at the point of contact.

Observation of mycelial mats from zone of interaction in dual culture plate between pathogen and antagonist under microscope showed that *Trichoderma* spp. was interacting with Mp hyphae. Antagonist hyphae were observed to be growing towards Mp hyphae and coiled around the hyphae.

The biocontrol agent was observed to produce knob like structure called as haustoria. These haustorial knob like structures with penetration pegs, penetrate the host and finally dissolve the protoplasm and shrink the hyphae which may lead to lysis [22]. Mycoparasitism as a principle mechanism of biological control is favoured by many scientists [20, 22]. Mycoparasitism includes hyphal interaction and parasitism, and is the most vital mechanism of the fungal antagonist to give protection to the plants against the pathogen attack. The growth of the mycoparasite towards the pathogen indicates a positive tropism probably, chemotropism of the parasite towards the host [21]. *Macrophomina phaseolina* was comparatively less inhibited by all *Trichoderma* species by the production of volatile compounds [13].

The results of the present study showed that among the species tested for antagonistic activity, only *Trichoderma* spp. highly inhibited the growth of the Mp than two species of *Penicillium* viz. *P. aurantiogriseum*, *P. islandicum* which showed no growth inhibition of the pathogen. It has also observed earlier that antagonistic fungi are specific in their antagonistic activity against specific fungi [23]. Antagonism by *Trichoderma* spp. against a range of soil borne plant pathogens has been reported earlier [24, 25, 26, 27]. *Trichoderma* species are the most commonly used bio control agents that showed effective antagonistic activity against plant pathogenic fungi.

CONCLUSION

Plant diseases caused by pathogenic fungi constrain the yields. In agriculture, farmers still depend on the use of chemical fungicides to control plant diseases. However, misuse of these synthetic chemicals cause hazardous to both environment and health. The alternative method for replacement of chemical fungicides has lead to the use of biological control agents. Biocontrol of soil borne pathogens is met by the introduction of microorganisms. Microorganisms that grow in the rhizosphere are ideal for use as biocontrol agents.

Our recent studies proved that *Trichoderma* spp. have the potential to control *Macrophomina phaseolina in vitro* to the extent of 77.77%. *Penicillium* spp. studied showed least antagonistic property. The potential use of these biocontrol agents can be improved by isolation, formulation and application methods, particularly in the field.

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