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IDENTIFICATION OF ANTAGONISTIC ACTIVITY OF *TRICHODERMA* SP. ISOLATES AGAINST SOIL BORNE PLANT PATHOGENS *IN VITRO*

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KEYWORDS

T. harzianum
S. rolfsii
R. solani
A. flavus

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ABSTRACT

The *Trichoderma* isolates showed significant reduction of radial diameter of all the pathogens in dual culture tested over control. The antagonist *T. harzianum* isolate (Mahakoshi) showed maximum extent of mycelial growth inhibition of *A. niger* (60.74 %), *F. oxysporum* f. sp. *ciceri* (59.84 %), *M. phaseolina* (59.07 %), and *S. rolfsii* (58.82 %). It was followed by *R. solani, A. flavus* and *C. capsici*, where 55.00, 53.33 and 49.19 per cent of growth inhibition. *T. viride* isolate (Targaon) showed significant reduction of radial diameter of all the pathogens in dual culture tested over control. The antagonist *T. viride* showed maximum extent of mycelial growth inhibition of *M. phaseolina* i.e. 58.70 per cent. It was followed by *A. niger, Fusarium,* and *R. solani*, where 52.96, 52.59 and 51.67 per cent of growth inhibition observed, respectively. However, *C. capsici,* and *S. rolfsii* showed 45.07 and 44.63 per cent growth inhibition, respectively. These prevalent and dominant *Trichoderma* isolates were used as biocontrol agents against various soil borne plant pathogens.

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INTRODUCTION

The relationship in which one species of an organism is inhibited or adversely affected by another species in the same environment is also known as "Antagonism". The species which adversely affects the other is said to be antagonistic. Many microbial antagonists have been reported to posses antagonistic activities against plant fungal pathogens, such as *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Bacillus subtilis, B. cereus*, *B. amyloliquefaciens*, *Trichoderma* spp., *Burkholderia cepacia*, *Saccharomyces* sp, *Gliocladium* sp., etc. (Suprapta, 2012).

Trichoderma species are commonly available in soil and root ecosystems. The activity of these useful species has been recognized from 1930 due to their biological antagonism against several plant pathogens. (Ranasingh, *et al.*, 2006). Antagonistic *Trichoderma,* reduces the growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions and enzyme secretion. In addition, the release of biocontrol agents into the environment has created a demand for the development of methods to monitor their presence or absence in soil.

The present work was conducted to identify the prevalent and dominant antagonist *Trichoderma* sp. isolates from different physiographic regions of western Maharashtra to use them as biocontrol agents against soil borne plant pathogens.

Antagonistic activities of *Trichoderma* sp. isolates

The biocontrol exercised by *Tricoderma* can occur by several antagonistic mechanisms such as nutrient competition, antibiotic production and mycoparasitism. Chitinases and â, 1-3 glucanases have been directly involved the mycoparasitism interaction between *Tricoderma* spp. and its host (Kubickek *et al.,* 2003).

Patale and Mukadam (2011) tested the antagonistic activities of three *Trichoderma* species, *viz*. *T. viride*, *T. harzianum*, and *T. hamatum* against seven pathogenic fungi, namely *A.niger*, *A. flavus, Phytophthora* sp., *F. oxysporum*, *R. solani*, *Penicillium notatum*, and *Alternaria solani*. They found that all three species of *Trichoderma* suppressed effectively the growth of seven pathogenic fungi.

Mishra *et al.,* (2011) invented the *T. viride* isolate (Tr 8) which showed 70, 68.2, 70, 73.3, 69.3 and 70.1 per cent growth inhibition against *R. solani, S. rolfsii, M. phaseolina, A. alternata, F. solani and C. capsici,* respectively. The cell free culture filtrate of *T. viride* Tr 8 showed 61.5, 58.32, 63.45, 62.62 per cent radial growth at 10 per cent concentration against *R. solani, S. rolfsii, M. phaseolina, C. capsici,* respectively. While, 20 per cent concentration showed 100 per cent mycelial growth inhibition.

Rekha (2012) isolated fortyfour isolates of *Trichoderma* (Tri-1 to Tri-44) against *Sclerotium rolfsii* was done through dual culture technique for their efficacy to reduce mycelial growth and formation of sclerotial bodies. Among the 44 tested isolates 10 isolates viz., Tri-8, Tri- 13, Tri- 15, Tri- 16, Tri- 19, Tri-23, Tri-27, Tri- 29, Tri- 41 and Tri- 44 were found to be efficient in reducing both mycelial growth and formation of sclerotial bodies by the pathogens.

Moqdad (2014) studied the antagonistic capability of *T. harzianum* isolates against soil borne pathogenic fungi *viz. Acremonium* sp., *Alternaria* sp., *Aspergillus* sp., *Penicillium* sp., *Pythium* sp., *Rhizoctonia* sp. and *Verticillium* sp. The results indicated that both isolated that *T. harzianum* inhibited the growth of all pathogenic fungi at varying degrees and *T. harzianum* isolate 2 inhibited the growth of pathogens (45.99 %) more than *T. harzianum* 1 (41.08 %).

Sinha and Padamin (2015) studied the antagonism of *Trichoderma* spp. against sheath blight of rice caused by *Rhizoctonia solani in vitro* in Manipur and reported the volatile compounds produced from the nine *Trichoderma* isolates showed inhibition of 35.33 to 79.53% of mycelial growth of *Rhizoctonia solani*.

MATERIALS AND METHODS

Collection of soil samples

A total of 56 soil samples (0-15 cm depth) were collected by using GPS technique as per the soil series prescribed by Challa *et al.* (1999) from different physiographic regions of western Maharashtra viz., Western Konkan Coast, Western Ghats (Sahyadri) and North Deccan Plateau (Upper, Lower and Metamorphic) so as to study the variability of plant pathogens and antagonist microorganisms.

Method of isolation plant pathogens and antagonists

The plant pathogens and antagonists were isolated from soil samples collected from western Maharashtra by the soil dilution plate technique and spread plate method (Johnson & Curl, 1972) which is briefly described here.

Ten gram of representative soil sample was transferred to an Erlenmeyer flask containing 100 ml sterile distilled water and mixed thoroughly by shaking for 5 to 10 minutes. 10 ml samples were immediately drawn from the suspension in motion and transferred to 90 ml sterile water blank and shaken for one minute. From each suspension serial dilutions were prepared to obtain the desired dilution. One ml of the final dilution was transferred aseptically to a Petri dish and 15-18 ml of medium was added to the Petri dish. Media used for isolation were Potato dextrose agar and *Trichoderma* selective medium (TSM).

The dish was rotated by hand in different directions so as to disperse it uniformly in each media. When the medium was solidified, the Petri dishes were incubated at 28 ± 2^{o}C for 7 days, before they were taken for counting their populations. Single colonies of each microorganisms were picked up, numbered and maintained on respective agar medium slants for further studies.

Eight *Trichoderma* spp. isolated from the soils of western Maharashtra were tested for their biocontrol potential against soil borne pathogens by dual culture technique.

The *Trichoderma* isolates were used against *A. niger, A. flavus, C. capsici, F. oxysporum* f. sp. *ciceri*, *M. phaseolina*, *S. rolfsii* and *R. solani in vitro*. Among them *T. harzianum* isolate (Mahakoshi) and *T. viride* isolate (Targaon) showing antagonism with fast and fluffy growth used for antagonism study.

Dual culture technique

Antagonistic activity of the *Trichoderma* sp. isolates against test pathogen was determined by "dual culture" technique (Dennis and Webster, 1971). All antagonistic pathogen combinations were examined on 20ml of PDA in 90 mm Petriplates, with four replicate plates per treatment. For dual culture technique, a mycelial plug (0.5cm in diameter), was taken from actively growing 3 day old culture of the test pathogens and *Trichoderma* sp. isolates placed 80 mm apart from each other on the PDA. For control treatments, a plug of test pathogen was placed on the PDA medium. The plates were incubated at 28 ± 1^{o} C. Observations on the antagonistic activities of bioagent on test pathogens were recorded after every 24 hr for 5 days. When the inhibition zone was formed, it was expressed as percentage inhibition and was calculated by the following formula (Edington *et al*., 1971).

$$\text{Inhibition \%} = \frac{C - T}{T} \times 100$$

Where,

C = Radial growth measurement of the pathogen in control (mm)

T = Radial growth of the pathogen in the presence of bioagent (mm).

RESULTS AND DISCUSSION

In vitro antagonism of *T. harzianum* isolate (Mahakoshi) against soil borne plant pathogens

T. harzianum isolate (Mahakoshi) was evaluated for antifungal

Table 1: *In vitro* antagonism of *Trichoderma harzianum* isolate (Mahakoshi) against soil Borne Plant pathogens

Sr. No.	Soil borne plant pathogens	Mycelial growth of pathogen indual culture*(mm)	Mycelial growth of pathogenin control*(mm)	Mycelial Growth Inhibition (%)
1	*A. flavus*	42.00	90.00	53.33
2	*A. niger*	35.33	90.00	60.74
3	*C. capsici*	26.17	51.50	49.19
4	*F. oxysporum* f. sp. *ciceri*	34.67	86.33	59.84
5	*M. phaseolina*	36.83	90.00	59.07
6	*R. solani*	40.50	90.00	55.00
7	*S. rolfsii*	37.33	90.00	58.82
	S.Em ±	1.27	-	1.75
	CD	3.86	-	5.29

*Each value is mean of three replications.

Table 2: *In vitro* antagonism of *Trichoderma viride* isolate (Targaon) against soil borne plant pathogens.

Sr. No.	Soil borneplant pathogens	Mycelial growthof pathogen in dual culture* (mm)	Mycelial growth ofpathogen indual culture*(mm)	Mycelial Growth Inhibition (%)
1	*A. flavus*	52.17	90.00	42.04
2	*A. niger*	42.33	90.00	52.96
3	*C. capsici*	32.50	59.17	45.07
4	*F. oxysporum* f. sp. *ciceri*	42.67	90.00	52.59
5	*M. phaseolina*	37.17	90.00	58.70
6	*R. solani*	43.50	90.00	51.67
7	*S. rolfsii*	49.83	90.00	44.63
	S.Em ±	1.05	-	1.37
	C.D	3.17	-	4.17

*Each value is mean of three replications.

Plate 1. Antagonism of *Trichoderma harzianum* isolate (Mahakoshi) against soil borne Plant pathogens

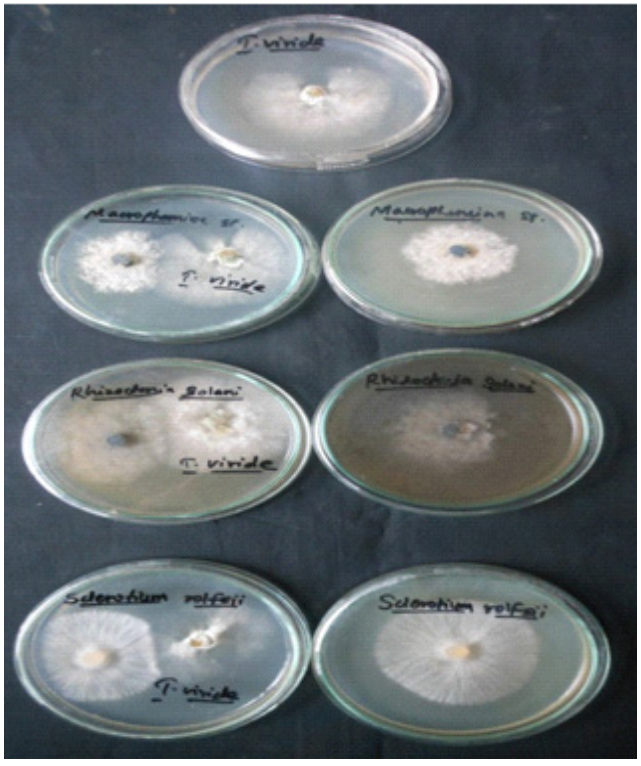

Plate 2: Antagonism of *Trichoderma viride* isolate (Targaon) against soil borne plant pathogens

activity against seven soil borne plant pathogens i.e. *A. niger, A. flavus, C. capsici, F. oxysporum* f. sp. *ciceri, M. Phaseolina*, *S. rolfsii* and *R. solani*.

The results presented in Table 1 (Plate 1) revealed that *T. harzianum* isolate (Mahakoshi) showed significant reduction of radial diameter of all the pathogens in dual culture tested over control.

The antagonist *T. harzianum* showed maximum extent of mycelial growth inhibition of *A. niger* (60.74 %), *F. oxysporum* f. sp. *ciceri* (59.84 %), *M. Phaseolina* (59.07 %), and *S. rolfsii* (58.82 %). It was followed by *R. solani, A. flavus* and *C. capsici*, where 55.00, 53.33 and 49.19 per cent of growth inhibition was recorded, respectively.

The zone of inhibition was also observed in case of all the pathogens in dual culture.

In vitro antagonism of *Trichoderma viride* isolate (Targaon) against soil borne plant

Pathogens

T. viride isolate (Targaon) was evaluated for antifungal activity against seven soil borne plant pathogens *i.e. A. niger, A. flavus, C. capsici, F. oxysporum* f. sp. *ciceri, M. phaseolina, S. rolfsii* and *R. solani*. The results in Table 2 (Plate 2.) revealed that *T. viride* isolate showed significant reduction of radial diameter of all the pathogens in dual culture tested over control. The antagonist *T. viride* showed maximum extent of mycelial growth inhibition of *M. phaseolina* i.e. 58.70 per cent.

It was followed by *A. niger, Fusarium,* and *R. solani*, where 52.96, 52.59 and 51.67 per cent of growth inhibition observed, respectively. However, *C. capsici,* and *S. rolfsii* showed 45.07 and 44.63 per cent growth inhibition,

respectively. While, *A. flavus* recorded lowest (42.04 %) of growth inhibition among all pathogens tested. The zone of inhibition was also observed in case of *A. niger, F. oxysporum* f. sp. *ciceri, M. phaseolina, R. solani* and *S. rolfsii* in dual culture.

These results were supported with the findings of Patale and Mukadam (2011) who tested the antagonistic activities of three *Trichoderma* species, *viz., T. viride*, *T. harzianum*, and *T. hamatum* against seven pathogenic fungi namely *A. niger*, *A. flavus, Phytophthora* sp., *F. oxysporum*, *R. solani*, *Penicillium notatum*, and *Alternaria solani*. They found that all three *Trichoderma* spp. suppressed effectively the growth of seven pathogenic fungi.

These findings are in close agreement with the findings of Mishra *et al.,* (2011) who invented the *T. viride* isolate (Tr 8) that showed 68.2 to 73.3 per cent growth inhibition against *C. capsici, F. solani A. alternata, M. phaseolina, S. rolfsii,* and *R. solani,* respectively.

The similar results on the antagonism *Trichoderma* spp. were confirmed by the earlier research workers against *Acremonium* sp., *Alternaria* sp., *Aspergillus* sp., *Penecillium* sp., *Pythium* sp., *Rhizoctonia* sp. and *Verticillium* sp. (Moqdad, *et al.,* 2014).

Sinha and Padamin (2015) in the *invitro* experiment of antagonism of *Trichoderma* spp. against sheath blight of rice caused by *Rhizoctonia solani* in Manipur showed that all the nine *Trichoderma* spp. isolates had potential to inhibit the mycelial growth of *R. solani*.

Thus the results are in conformity with the research work carried out by earlier research workers.

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