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In vitro evaluation of *Trichoderma* species for virulence efficacy on *Botryodiplodia palmarum*

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ABSTRACT

Trichoderma species are well known for their antagonistic activity against number of phyto-pathogens. They restrict the growth of pathogens by variety of means like parasitism, release of antibiotic volatile and non-volatile metabolites. The studies aimed to screen and evaluate the growth inhibition potential of six *Trichoderma* species against *Botryodiplodia palmarum* under laboratory conditions. The results revealed 37.56-61.95% growth inhibition of *B. palmarum* in dual culture, 12.59-43.46% and 4.38-78.08% respectively by the release of volatile and non-volatile metabolites. The growth inhibition was increased with period of incubation and concentration of culture filtrate of *Trichoderma* species in the culture media. *Trichoderma harzianum* was most effective among the selected species.

Keywords: Biocontrol; Antagonist; *Trichoderma*; *Botryodiplodia palmarum*.

1. INTRODUCTION

Plants are the backbone of life on earth and their productivity is reduced by variety of biotic and abiotic pressures. Poplars are easy to cultivate and constitute an important component of forestry and agroforestry systems for the livelihood of small-scale farmers [1]. The diseases, in particular due to fungi cause huge loss to the crops. *Botryodiplodia palmarum* is known to cause nursery diseases of poplars in northern states of India [2-4]. Different approaches are being implemented to prevent and manage plant diseases. Agrochemicals are contri-buting significantly in disease management and improvement of crop productivity, but their exces-sive use has resulted in environmental pollution and development of resistance in pathogens. As per the WHO estimates, approximately 750,000 people fall ill every year due to pesticide poisoning and about 14,000 of them die due to agony [5]. As a result of growing concerns about health and environmental problems associated with pesticides, it is need of hour to develop eco-friendly approaches for the management of plant diseases.

Trichoderma species are well known bio-control agents for the management of phytopatho-gens. The previous laboratory and field experiments projected *Trichoderma* species as plant growth promoter and antagonistic to plant pathogens [6-11]. Therefore, application of *Trichoderma* species having significant antagonistic potential against a wide range of phytopathogenic fungi seems to be better alternative to the synthetic fungicides. *Trichoderma* species are successful biocontrol agents due to their rapid multiplication

and ability to tolerate the harsh conditions beside their antagonistic potential. They also overcome the phytopathogens by competing for nutrients and space. *Trichoderma* species harm the pathogens by direct parasitism or by the release of volatile and non-volatile antibiotic metabolites into their vicinity. Perello et al. [7] reported 50-70% growth inhibition of *Drechslera triticeripentis* by *Trichoderma* species in the range dual cultures. Sharma [12] evaluated 18 isolates of *Trichoderma* for their biocontrol activity against *Fusarium oxysporum f.* sp. *pisi* and observed initial counter inhibition of *Trichoderma* and *Fusarium* in all the 18 dual culture sets at varying degree. Ambuse [10] recorded up to 80% growth inhibition against resistant isolates of *Alternaria tenuissima* in dual cultures by *Trichoderma* species.

Trichoderma species are also known to release gaseous metabolites, which are toxic to other group of fungi. Its major advantage is that the toxic substances may diffuse through air filled pores thus actual contact between antagonist and pathogen is not necessary. Rathore et al. [13] recorded reduction of growth and vacuole formation in the hyphae of *F. solani* by the volatile compounds of *T. viride*. Doi and Mori [14] observed adverse effect on the hyphal tips of *Lentinus lepideus* and *Coriolus versicolous* by the volatile compounds of *T. viride.* Amin et al. [9] also observed mycelial growth inhibition of *F. oxysporum* and sclerotial production in *S. rolfsii* and *S. sclerotiorum* by the volatile compounds of *T. viride.* Anoop et al. [15] also recorded considerable growth suppression of *Pythium aphanidermatum* by *Trichoderma* species in dual culture as well by the release of volatile compounds.

Beside the volatile compounds, *Trichoderma* species also release non-volatile antibiotic metabolites. Kexiang et al. [16] recorded growth suppression of *Botryosphaeria berengeriana* by non-volatile substances released by *T. harzianum* and *T. atroviridi*. Krupke et al. [17] showed that *A. bisporus* colonies were suppressed by diffusible metabolites of *T. harzianum*. Rajendiran et al. [18] recorded up to 64% growth inhibition of *Aspergillus* species by non-volatile compounds of *Trichoderma* spp. released into liquid medium. Tapwal et al. [19] also reported *Trichoderma viride* as potential antagonist under laboratory conditions against the species of *Rhizoctoia, Alternaria, Curvularia,* and *Fusarium* species. Kushwaha and Verma [20] recorded significant antimycotic activity of *Trichoderma* spp. against many crop pathogens including *Colletotrichum, Alternaria, Paracercospora* and *Fusarium* species. *Trichoderma* species are known to restrict the phytopathogens by variety of means, therefore the present study was undertaken to investigate the virulence efficacy of *Trichoderma* species against *Botryodiplodia palmarum* under *in-vitro* conditions.

2. MATERIAL AND METHODS

Six *Trichoderma* species (*T. harzianum, T. koningii, T. longibrachiatum, T. piluliferum, T. virens* and *T. viride*) and pathogen (*Botryodiplodia palmarum*) were obtained from Forest Pathology Division, Forest Research Institute, Dehradun.

2.1 Evaluation of *Trichoderma* spp. for antagonism against *B. palmarum* in dual culture

Five mm mycelial discs of antagonist and pathogen were inoculated 4 cm apart on PDA in Petri plates. In control only disc of pathogen was inoculated in similar manner. The plates were incubated at 25±1°C. Radial growth of the pathogen was measured on fifth day of incubation and compared with control [21].

Percent growth inhibition was determined by following formula:

Percent inhibition = (A1-A2)/A1*100

Where A1 is area covered by pathogen in control and A2 is area covered by pathogen in dual culture.

2.2 Virulence efficacy of volatile compounds released by *Trichoderma* species on the growth of *B. palmarum*

Antagonist and pathogen were inoculated in different Petri plates on PDA and the Petri plates were sealed with adhesive tape (parafilm) keeping antagonist in lower and pathogen in the upper Petri plate and incubated at 25±1°C [22]. In control, Petri plate containing pathogen was inverted over the Petri plate containing the medium only. Two sets of experiments were laid, in first set, the antagonist

and pathogen were of same age while in second set the antagonist culture was one day older than pathogen. The colony diameter of pathogen was measured on the fifth day and compared with control.

2.3 Virulence efficacy of non-volatile compounds released by *Trichoderma* species on the growth of *B. palmarum*

Poisoned food technique [23] was followed to evaluate the effect of non-volatile compounds released by *Trichoderma* spp. on the growth of *B. palmarum*. *Trichoderma* spp. were grown on potato dextrose broth up to 4 weeks, at the interval of one week, the media containing fungal antagonist was filtered through Whatman-I filter paper, a requisite amount of the filtrate was mixed with potato dextrose agar (PDA) to get the desired concentrations of 10, 20 and 30% and autoclaved. Five mm mycelial discs of pathogen were inoculated on PDA amended with culture filtrate and control (without culture filtrate). Colony diameter of pathogen was measured on fifth day of incubation and compared with control.

3. RESULTS AND DISCUSSION

3.1. Interaction between pathogen and antagonists in dual culture

Trichoderma species have restricted the growth of *B. palmarum* in the range of 37.56-61.95% and an inhibition zone has been observed at the point of contact between antagonist and pathogen. On the fifth day of incubation maximum growth inhibition (61.95%) was exhibited by *T. harzianum*, followed by *T. virens* (55.36%), *T. viride* (50.48%), *T. koningii* (45.60%), *T. longibrachiatum* (39.02%) and minimum (37.56%) by *T. piluliferum* (Table 1).

The growth inhibition on dual cultures has been attributed to inhibitory substances released by one or both organisms, competition, and also mechanical obstruction and hyperparasitism [21]. Sangeeta et al. [24] isolated 12 *Trichoderma* species from the soil of banana plantation and found them inhibitory against two banana crown rot pathogens (*B. theobromae, C. musae*). Microscopic examinations on the interaction of *T. viride* and *T. harzianum* isolates with *B. theobromae* and *C. musae* revealed direct parasitism by antagonists by coiling around the mycelium of pathogen. Although in present study, a zone of inhibition was recorded at the point of contact between *Trichoderma* spp. and *B. palmarum* but the coiling of antagonist and pathogen hyphae was not recorded. It was also observed that the antagonist overgrew the pathogen, if culture plates were inoculated for longer periods. *Trichoderma* species were reported as potential antagonists against variety of phytopathogens [21, 25]. Ambuse et al. [10] also recorded upto 80% growth inhibition by *Trichoderma* spp. against sensitive and resistant isolates of *Alternaria tenuissima* in dual culture.

3.2 Virulence efficacy of volatile compounds released by *Trichoderma* species on the growth of *B. palmarum*

The volatile compounds released by *Trichoderma* species restricted the growth of *B. palmarum* in the range of 12.59-43.46% (Table 2). When the antagonists and pathogen were of same age, the volatile compounds released by *Trichoderma* species have inhibited the growth of pathogen in the range of 12.59-22.22% and it was in the range of 28.26-43.46%, when the antagonist cultures were one day older than pathogen. Maximum growth inhibition of *B. palmarum* was recorded by *T. harzianum.*

The antagonist growing in the lower culture plate may have released volatile metabolites, which may have inhibited the growth of the pathogen growing in the top Petri plate. *Trichoderma* spp. have variable growth rate and therefore the amount of antibiotic metabolites release by them also vary. The higher growth inhibition in one day older antagonist may be due to higher growth and subsequent more release of antibiotic metabolites. The major advantage of antibiosis in case of volatile compounds is that the toxic substances released by the antagonists may diffuse through air filled pores in soil and helps in checking the root rot pathogen without establishing actual physical contact with the pathogen. Dennis and Webster [22] reported the influence of volatile metabolites of *T. harzianum* on the growth of *Rhizoctonia solani* and other fungi.

Table 1. Growth inhibition (%) of *B. palmarum* by *Trichoderma* species on the fifth day of incubation in dual culture.

***Trichoderma* species**	**Growth Inhibition (%)**
T. harzianum	61.95
T. koningii	45.60
T. longibrachiatum	39.02
T. piluliferum	37.56
T. virens	55.36
T. viride	50.48

Table 2. Effect of volatile compounds released by *Trichoderma* species on the growth of *B. palmarum.*

***Trichoderma* spp.**	**Growth Inhibition (%)**	
	Age of Antagonist	
	Same age	**One day**
T. harzianum	22.22	43.46
T. koningii	13.70	32.86
T. longibrachiatum	16.29	28.26
T. piluliferum	17.40	32.86
T. virens	13.70	31.80
T. viride	12.59	39.92

Gveroska and Ziberoski [26] observed inhibitory activity of volatile compounds of *Trichoderma* species on the growth of *Alternaria alternata*. Ng et al. [27], also observed releases of volatile compounds by *Trichoderma* species beside the hydrogen cyanide production and plant growth-promotion properties.

3.3 Virulence efficacy of non-volatile compounds released by *Trichoderma* species on the growth of *B. palmarum*

The culture filtrate of *Trichoderma* species have restricted the growth of *B. palmarum* in the range of 4.38-78.08% and rate of inhibition increased with the age of antagonist and concentration of culture filtrate in amended media (Table 3). Wide-ranging growth inhibition by different antagonists may due to their variable growth rate in the culture media. At 30% concentration of culture filtrate in PDA the growth inhibition was in the range of 15.39-78.08%, followed by 20% concentration in PDA (8.80-56.15%) and minimum 4.38-41.10% at 10% concentration of culture filtrate. *T. harzianum* was found most effective antagonist among all treatments.

Table 3. Effect of non- volatile compounds released by *Trichoderma* species on the growth of *B. palmarum.*

Age of antagonist	***Trichoderma* spp.**	**Growth inhibition (%) at different concentrations of culture filtrate**		
		10%	**20%**	**30%**
One week	*T. harzianum*	12.10	24.16	35.17
	T. koningii	7.68	12.10	20.33
	T. longibrachiatum	14.27	17.57	27.46
	T. piluliferum	7.68	13.18	15.39
	T. virens	9.89	15.39	19.70
	T. viride	4.38	8.80	15.39
Two weeks	*T. harzianum*	26.40	37.50	50.00
	T. koningii	21.25	32.31	37.50
	T. longibrachiatum	24.16	36.46	44.68
	T. piluliferum	19.70	28.90	39.59
	T. virens	15.39	25.39	29.78
	T. viride	19.33	26.92	36.46
Three weeks	*T. harzianum*	34.61	45.56	63.33
	T. koningii	32.23	41.10	54.46
	T. longibrachiatum	28.45	42.56	56.66
	T. piluliferum	28.90	38.33	55.56
	T. virens	32.23	34.16	47.80
	T. viride	30.76	36.46	46.66
Four weeks	*T. harzianum*	41.10	56.15	78.08
	T. koningii	38.90	46.66	63.84
	T. longibrachiatum	32.23	47.80	67.80
	T. piluliferum	33.34	43.33	60.25
	T. virens	34.16	47.80	54.88
	T. viride	36.66	45.56	51.15

While the antagonists were growing in culture media have utilized the nutrients and may have released some non-volatile secondary metabolites in the growing media which may be responsible for growth inhibition of pathogen. In similar experiments, Mishra et al. [28] observed that the non-volatile compounds of *Trichoderma* species have inhibited the growth of *Rhizoctonia, Fusarium, Alternaria, Colletotrichum,* etc. Eziashi et al. [8]

also tested metabolites released from *Trichoderma viride, Trichoderma polysporum, Trichoderma hamatum,* and *Trichoderma aureoviride,* in a culture medium against *Ceratocystis paradoxa,* a casual organism of black seed rot in oil palm sprouted seeds. Tapwal et al. [29] also reported *Trichoderma viride* as a potential antagonist under laboratory conditions against the species of *Rhizoctonia, Alternaria, Curvularia,* and *Fusarium* species.

It is evident form the results that the *Trichoderma* species have restricted the growth of *B. palmarum* in dual culture as well by the release of volatile and non-volatile compounds. The growth inhibition potential of *Trichoderma* species increased with its age in culture media as well by at higher concentration of culture filtrate. Among tested *Trichoderma* species, *T. harzianum* was most effective in all treatments and can be evaluated in nursery and field conditions before developing the formulations.

AUTHORS CONTRIBUTION

AT: Overall design and execution of research work and paper writing; HP: Laboratory experiment and observations. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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