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Full Length Article

Effect of Culture Medium on Herbicidal Potential of Metabolites of *Trichoderma* Species against *Parthenium hysterophorus*

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Abstract

Laboratory and pot trials were conducted to investigate the herbicidal potential of metabolites of *Trichoderma* spp. viz. *T. pseudokoningii*, *T. harzianum*, *T. viride* and *T. reesei* against parthenium (*Parthenium hysterophorus* L.) weed. Fungal metabolites were prepared in malt extract broth and M-I-D medium. In laboratory bioassays, parthenium seed were sown on filter papers in Petri plates (9-cm diameter) and 3 mL of original and 50% diluted metabolites of the test *Trichoderma* spp. were applied. Metabolites of all the four fungal species showed herbicidal action. The highest herbicidal activity was observed due to metabolites of *T. harzianum*. Fungal metabolites prepared in M-1-D medium generally showed greater inhibitory potential against seed germination and seedlings root/shoot growth as compared to the metabolites prepared in the other medium. For foliar spray bioassays, pot grown parthenium seedling at 1- and 2-week growth stages were sprayed with original fungal metabolites. Foliar spray was carried out three times with five days of intervals. In these pot trials, metabolites of all the four fungal species reduced shoot and root growth significantly in 1-week old parthenium plants. Metabolites prepared in M-1-D medium showed greater herbicidal effect than those formed in malt extract broth. It is concluded that metabolites of *Trichoderma* species, particularly prepared in M-1-D medium can be exploited for the management of parthenium weed.

Keywords: Alternative herbicides; Fungal metabolites; *Parthenium hysterophorus; Trichoderma*

Introduction

Parthenium, family Asteraceae, is an invasive weed native to America and has become widespread in many regions of the world (Navie *et al.*, 1996). It has the capability to form massive monocultural stands with little or no other plant species in the neighborhood, and is responsible for many problems related to environment and agriculture including loss of crop yield through allelopathy and competition, shortage of fodder, depletion of biodiversity, and health related problems for humans and stock (Evans, 1997). The invasive nature of parthenium is due to various allelochemicals in its different parts especially the parthenin (Reinhardt *et al.*, 2009). Some chemical herbicides namely atrazine, glyphosate, chlorimuron ethyl, ametryn, bromoxynil, metsulfuron methyl and metasulfuron have proved very successful in controlling parthenium weed (Mishra and Bhan, 1994; Javaid, 2007; Goodall *et al.*, 2010; Shahzad *et al.*, 2012). However, due to increased public concerns on environmental issues, there is a need of alternative weed management systems based on natural compounds and is less pesticides dependent (Kovalchuk *et al.*, 2008). Recent studies have revealed that naturally occurring compounds in plants (Javaid *et al.*, 2006, 2010) and fungi (Javaid and Adrees, 2009; Javaid, 2010; Singh *et al.*, 2010) have the potential to be used as bioherbicides to manage the invasive parthenium weed.

Trichoderma spp. are soil fungi known for their antagonistic behavior against numerous soil-borne bacterial, fungal and invertebrates plant pathogens (Verma *et al.*, 2007). However, studies concerning the herbicidal activity of these fungi are very rare and are generally limited only to *Trichoderma virens* (Hutchinson, 1999; Heraux *et al.*, 2005a, b). Recently, Javaid and Ali (2011) reported herbicidal activity of some *Trichoderma* spp. from Pakistan against *Rumex dentatus*, a problematic weed of wheat. The present research work was intended to assess the effect of culture medium on herbicidal potential of metabolites of four species of *Trichoderma* viz. *T. viride, T. harzianum, T. pseudokoningii* and *T. reesei* against parthenium.

Materials and Methods

Preparation of Fungal Metabolites

Two liquid growth media viz. malt extract and M-1-D were used for the preparation of fungal metabolites. Hundred milliliters of malt extract broth was autoclaved in 250-mL volume flasks. After cooling at room temperature, flasks were inoculated with discs of the *Trichoderma* test species separately. Flasks were incubated on a shaker at 150 rpm at 25°C for 28 days. Thereafter, sterilized muslin cloth was

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used to filter the fungal metabolites followed by Whatman filter paper No. 1 and finally through millipore filterpapers. The filtrates were stored at 4°C and generally used within one week of filtration.

The second growth medium was M-1-D with some modification. It consisted of NaH_2PO_4, 0.14 mM; $MgSO_4$, 3.0 mM; $ZnSO_4$, 8.7, uM; KNO_3, 0.79 mM; $Ca(NO_3)_2$, 1.2 mM; KCI, 0.87 mM; $MnSO_4$, 30, uM; $FeC1_3$, 7.4, uM; ammonium tartrate, 27.1 mM; sucrose, 87.6 mM and KI, 4.5 µM. pH of the medium was adjusted to 5.5 using 0.1 M HC1 (Pinkerton and Strobel, 1976). Inoculation of *Trichoderma* spp., incubation and filtration of the culture filtrates were as described for malt extract medium.

Laboratory Bioassays

Petri plates (9-cm diameter) were lined with sterilized filter papers. Three milliliters of original and diluted (50%) metabolites of the test fungal species prepared in malt extract and M-1-D media were poured in each plate. Three mililitrers of sterilized distilled water was used in control treatments instead of fungal metabolites. Seeds of parthenium were collected from University of the Punjab, Quaid-e-Azam campus Lahore, Pakistan. Fifteen seeds of parthenium weed were placed on moistened filter papers in each Petri plate. Each treatment was replicated three times. The plates were placed in a completely randomized design in a growth room set at 20±2°C and a light period of 10 h daily. Data regarding germination, shoot and root length, and fresh weight of the plants were recorded (Javaid and Ali, 2011). Seedlings were harvested after ten days.

Foliar Spray Bioassays

Twelve-centimeter deep plastic pots were filled with 350 g of sandy loam textured soil. The soil had pH 7.8, 0.036% nitrogen, 6.31 mg kg^{-1} available phosphorus and 100 mg kg^{-1} available potassium. In each pot, 10 weed seeds were sown. After germination, extra plants were removed and six uniform plants were maintained in each pot. Metabolites of the test fungal species were prepared in malt extract and M-1-D medium. For foliar spray bioassays, pots were divided into two groups i.e., 1-week and 2-week old. The original fungal metabolites prepared in malt extract and M-1-D medium were sprayed three times with an interval of 5 days. Experiment was conducted in a completely randomized design under natural conditions. Plants of control treatment were sprayed with distilled water. Harvest was taken 40 days after sowing and data regarding root and shoot length and dry biomass were recorded.

Statistical Analysis

Data were analyzed by ANOVA followed by Duncan's Multiple Range Test ($P \leq 0.05$) to separate treatments means (Steel and Torrie, 1980).

Results

Laboratory Bioassays

Analysis of variance showed that there was significant effect of growth medium (G), *Trichoderma* species (S) and concentration (C) for germination, seedling shoot and root length, and plant biomass. All the interactive effects of G × S, G × C and G × S × C were also significant for germination and various parameters of plant growth. However, the effect of S × C was non-significant for root length (Table 1).

Data (Table 2) revealed that original metabolites (100%) of the test fungal species significantly reduced germination of parthenium seeds. Similarly, diluted metabolites (50%) of all fungal species except *T. pseudokoningii* also reduced the seed germination significantly. Metabolites of *T. harzianum* and *T. reesei* were more effective in suppressing the germination than metabolites of other two species. There was 4–37% and 32–53% reduction in seed germination due to diluted and original metabolites of different fungal species respectively (Table 2). Original, as well as diluted metabolites of different *Trichoderma* spp. significantly reduced shoot length of parthenium seedlings. Metabolites of *T. pseudokoningii* exhibited least herbicidal activity causing up to 44% suppression in shoot length over control. Conversely, metabolites of *T. harzianum* showed marked herbicidal activity resulting up to 85% reduction in shoot length. Metabolites of *T. reesei* and *T. viride* reduced shoot length up to 49% and 64% over control, respectively (Table 2). The adverse effect of original as well as diluted metabolites of all the four *Trichoderma* spp. was also significant on root length of parthenium seedlings. The highest herbicidal activity against root length was exhibited by culture filtrates of *T. viride* and the lowest one by filtrates of *T. pseudokoningii* (Table 2). The effect of various fungal metabolites on shoot biomass was significant. Metabolites of *T. harzianum* exhibited the highest herbicidal activity where 50% and 100% metabolites reduced the weed biomass by 48% and 77%, respectively. *T. pseudokoningii* exhibited the least herbicidal activity causing 22-56% reduction in weed biomass due to different concentrations of fungal metabolites. Metabolites of the other two fungal species reduced the weed biomass by 55–66% (Table 2).

The effect of fungal metabolites prepared in M-1-D medium on germination and seedling growth of parthenium is depicted in Table 2. Metabolites of all the four *Trichoderma* spp. except *T. viride* reduced germination of parthenium seeds significantly both in original and diluted concentration treatments. There was 0–71% reduction in germination of the weed seeds due to different concentrations of metabolites of the four *Trichoderma* spp. Both the concentrations of *Trichoderma* species metabolites significantly declined the shoot and root length as well as biomass of parthenium seedlings. In general, the effect of fungal metabolites prepared in M-1-D medium was more

Table 1: Analysis of variance for the effect of different concentrations of culture filtrates of four *Trichoderma* species prepared in malt extract medium and M-1-D medium, on germination and growth of parthenium in laboratory bioassays

Trait	df	Mean squares			
		Germination	Shoot length	Root length	Plant fresh wt.
Treatments	23	1859***	208***	213***	44***
Growth medium (G)	1	345*	193***	194***	38***
Trichoderma species (S)	3	2708***	94***	13**	5***
Concentration (C)	2	10662***	1866***	2199***	444***
G × S	3	832***	67***	24***	7***
G × C	2	514***	55***	53***	10***
S × C	6	1019***	24***	5 NS	2***
G × S × C	6	553***	18***	9**	4***
Error	72	64	1.1	2.56	0.12
Total	95				

*, **, ***, significant at P ≤ 0.05, 0.01 and 0.001, respectively; NS: Non significant

Table 2: Effect of culture filtrates of four *Trichoderma* species prepared in malt extract medium and M-1-D medium on germination and growth of parthenium in laboratory bioassays

Fungal species	Conc. (%)	Germination (%)	Shoot length (mm)	Root length (mm)	Plant fresh weight (mg/plant)
Malt Extract Medium					
Control	0	100 a	20.9 a	19.4 a	9.6 a
T. horzianum	50	79 b-d	7.9 fg	8.7 cd	4.9 c
	100	49 g	3.1 j	4.9 f-h	2.2 fg
T. psedokoningii	50	96 a	17.8 b	15.2 b	7.5 b
	100	83 bc	11.8 cd	7.2 de	4.4 d
T. ressei	50	63 ef	10.6de	9.2 c	4.3 d
	100	47 g	7.6 g	5.7 e-g	3.3 e
T. viridi	50	89 ab	11.8 cd	7.1 e	4.4 d
	100	68 de	8.8 fg	3.2 h-j	3.4 e
M-1-D Medium					
T. horzianum	50	60 e-g	3.9 ik	4.1 g-i	1.9 g
	100	52 fg	1.5 k	1.4 k	0.7 i
T. psedokoningii	50	55 fg	5.0 hi	4.4 f-h	2.3 f
	100	72 c-e	2.9 jk	1.5 k	0.9 i
T. ressei	50	69 de	5.7 h	5.7 e-g	4.5 d
	100	29 h	3.5 ij	1.6 jk	1.3 h
T. viridi	50	92 ab	13.0 c	5.9 ef	3.4 e
	100	100 a	9.4 ef	2.5 i-k	3.5 e

In a column, values with different letters show significant difference (P ≤ 0.05) as determined by Duncan's Multiple Range Test

severe than those prepared in malt extract medium. There was 70-92%, 38-93% and 53-93% reduction in root length shoot, length and biomass, respectively, due to metabolites of different *Trichoderma* spp. Metabolites of *T. harzianum* proved the best as a herbicide in laboratory bioassays (Table 2).

Foliar Spray Bioassays

Analysis of variance revealed that the effect of growth medium (G) and plant age (A) was significant (P ≤ 0.001) for biomass and length of both shoot and root (Table 3). However, the effect of *Trichoderma* spp. (S) was significant

Table 3: Analysis of variance for the effect of culture filtrates of four *Trichoderma* species prepared in malt extract medium and M-1-D medium, on shoot and root growth of parthenium in foliar spray bioassays

Trait	df	Mean squares			
		Shoot length	Shoot biomass	Root length	Root biomass
Treatments	23	1143***	1161***	9315***	132***
Growth medium (G)	1	9311***	1790***	51490***	336***
Trichoderma species (S)	3	9 NS	40 NS	3254**	18 NS
Plant age (A)	2	5704***	11395***	38662***	962***
G × S	3	32 NS	30 NS	2757*	65 NS
G × A	2	2633***	518**	22545***	92 NS
S × A	6	6 NS	55 NS	2785**	17 NS
G × S × A	6	23 NS	92 NS	933 NS	39 NS
Error	72	46	83	838	29
Total	95				

*, **, ***, significant at P ≤ 0.05, 0.01 and 0.001, respectively; NS: Non significant

(P ≤ 0.01) for root length only. Similarly, the effects of G × S and S × A were significant only for root length. The effect of G × A was significant for all the studied root and shoot growth parameters except root biomass. Conversely, the effect of G × S × A was insignificant for all the parameters (Table 3).

Foliar spray of all the fungal metabolites except *T. pseudokoningii* significantly declined the shoot length of 1-week old seedlings (Fig. 1). There was 30%, 25%, 29% and 20% suppression in shoot length due to metabolites of *T. reesei*, *T. harzianum*, *T. viride* and *T. pseudokoningii*, respectively. The effect of metabolites spray of all the *Trichoderma* spp. was non-significant on 2-week old seedlings (Fig. 1A). The adverse effect of all the fungal metabolites on shoot biomass was significant both in 1-week and 2-week old plant treatments. However, generally 1-week old plants were more vulnerable to fungal metabolites than the 2-weeks old ones. There was 49–79% and 28–53% reduction in shoot biomass in 1-week and 2-week old plants, respectively, due to metabolites of different *Trichoderma* spp. (Fig. 1B). Root length was significantly suppressed due to foliar spray of metabolites of *T. viride* and *T. reesei* in 1-week old seedlings. In 2-week old plants, metabolites of *T. reesi* significantly reduced the root length, while those of other fungi enhanced root length by 16–43% (Fig. 1C). Metabolites of all the four *Trichoderma* spp. significantly reduced root biomass in 1-week old plants by 30–68%. However, in 2-week old plants, only the metabolites of *T. pseudokoningii* and *T. reesei* exhibited significant adverse effect resulting in 33% and 53% reduction in root biomass, respectively (Fig. 1D).

Shoot length and biomass were significantly reduced by metabolites of all the four *Trichoderma* species in 1-week as well as 2-week old parthenium plants (Fig. 2). There was 68–86% and 87–99% suppression in shoot length, and 80–91% and 77–98% decline in shoot biomass due to metabolites of various *Trichoderma* spp. in 1- and 2-week old plants, respectively (Fig. 2A and B). Root growth was comparatively less susceptible to foliar spray than the

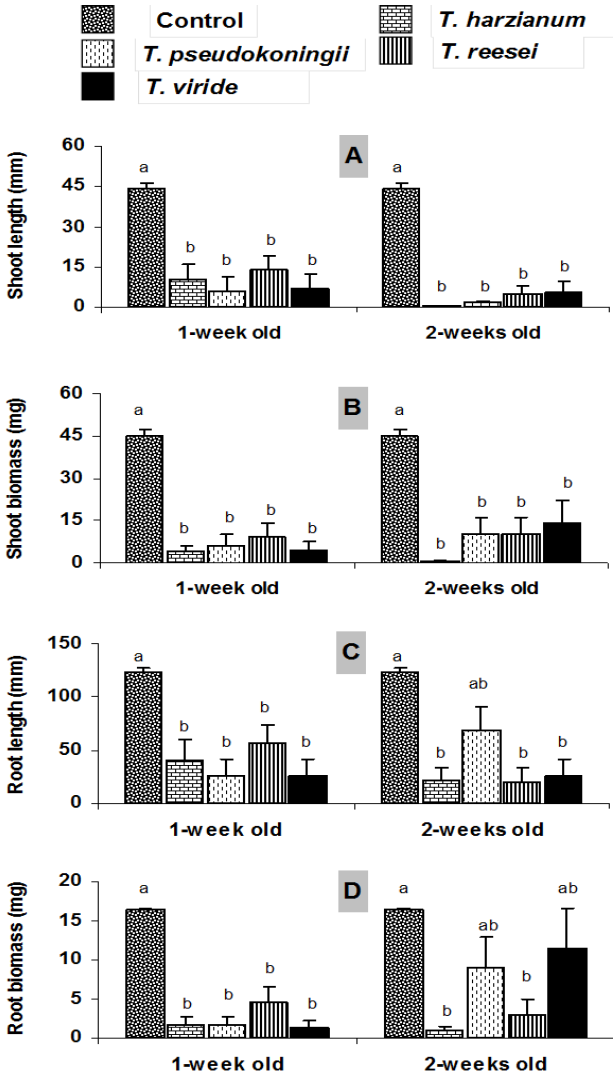

Fig. 2: Effect of foliar spray of cultural filtrates of four species of *Trichoderma* (in M-1-D medium) on growth of 1-week and 2-weeks old plants of parthenium. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference as determined by Duncan's Multiple Range Test at $P \leq 0.05$

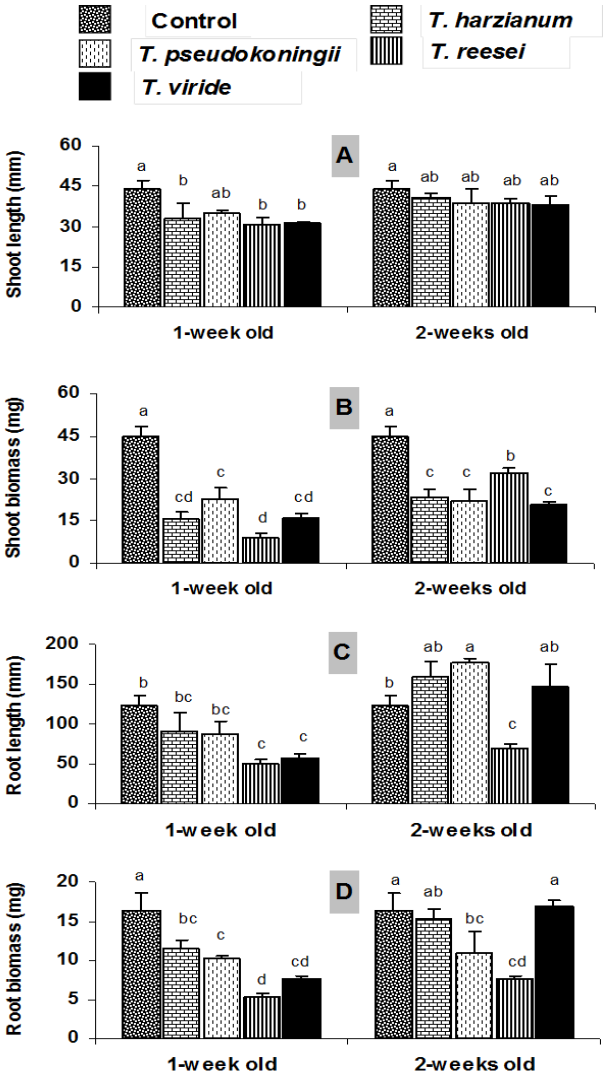

Fig. 1: Effect of foliar spray of cultural filtrates of four species of *Trichoderma* (in malt extract medium) on growth of 1-week and 2-weeks old plants of parthenium. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference as determined by Duncan's Multiple Range Test at $P \leq 0.05$

shoot growth. In 1-week old plants, there was a significant reduction in root length due the test fungal metabolites. In 2-week old plants, metabolites of all the fungal species except *T. pseudokoningii*, significantly suppressed root length. There was 54–79% and 44–84% decline in root length due metabolites of different *Trichoderma* spp. in 1- and 2-week old plants (Fig. 2C). Root biomass of 1-week old plants was significantly reduced by 72–92% due to different fungal metabolites. However, in 2-week old plants, only the effect of metabolites of *T. reesei* and *T. harzianum* was significant (Fig. 2D).

Discussion

Generally, previous studies established the role of *Trichoderma* spp. as biological control agent (Hanada *et al.*, 2009), and as source of commercial enzyme production (Kovacs *et al.*, 2009). The present research work reveals the potential of *Trichoderma* spp. as weed control agents. In this study, the four tested *Trichoderma* species showed herbicidal potential for the management of parthenium weed. These findings support the results of very few studies conducted previously in this research area (Hutchinson, 1999; Heraux *et al.*, 2005a). However, the previous studies were generally limited to the use of *T. virens* only (Hutchinson, 1999; Heraux *et al.*, 2005a, b). These fungi produce a variety of metabolites such as gliotoxin, viridiol, viridian, and gliovirin, which are toxic. Among these, viridiol is highly phytotoxic (Jones and Hancock, 1987) and could be responsible for herbicidal activity.

The metabolites of different *Trichoderma* spp. showed variable herbicidal activity against parthenium weed in laboratory. Generally, metabolites of *T. viride* and *T. pseudokoningii* exhibited comparatively lower herbicidal activity against parthenium as compared to the metabolites of other two fungal species. The variation in herbicidal activity among the four *Trichoderma* spp. species may be due to the presence of different types of secondary metabolites in different fungal species (Wang *et al.,* 2003; Zhou *et al.,* 2008; Eneyskaya *et al.,* 2009; Yang *et al.,* 2009). Metabolites of *T. harzianum* also exhibited pronounced activity in foliar spray pot trials. This fungal species is known to produce toxins which belong to the class trichothecene (Sivasithamparam and Ghisalberti, 1998). These are sesquiterpenoid epoxides and are known for their phytotoxic activity (Ueno, 1980; Harris *et al.,* 1999). The toxicity of these compounds is ascribed to their capability to hinder protein and DNA synthesis (McLaughlin *et al.,* 1977). A secondary metabolite Trichosetin is formed in *T. harzianum* culture that contains tetramic acid and is known to demonstrate phytotoxicity. *T. harzianum* also synthesizes another phytotoxic compound 6-pentyl-α-pyrone (Rocha *et al.,* 2006) that may be responsible for herbicidal activity.

Two growth media viz. malt extract and M-1-D were used for the preparation of *Trichoderma* spp. metabolites. In general, metabolites prepared in M-1-D medium exhibited greater herbicidal activity against germination and growth of parthenium in laboratory as well as foliar spray bioassays. The difference in herbicidal activity of the culture filtrates prepared in two different growth media was also highly prominent in pot trials where metabolites prepared in M-1-D medium severely affected the growth of parthenium seedlings. The variable herbicidal potential of the fungal metabolites prepared in different growth media could be due to the formation of different quantities of culture filtrates in different growth media (Zonno *et al.,* 2008).

In laboratory bioassays, generally the effect of various fungal metabolites was more severe on root growth as compared to shoot growth. Similar effect has also been reported for metabolites of *Alternaria alternata, Drechslera rostrata, D. australiensis, Fusarium solani* and *F. oxysporum* against parthenium (Adrees and Javaid, 2008; Javaid and Adrees, 2009). It is because the toxic ingredients are first absorbed by the root resulting in their abnormal growth.

In conclusion, four tested *Trichoderma* spp. contain compounds having herbicidal activity and can be used for parthenium management. However, *T. harzianum* has more potent herbicidal constituents than rest of the *Trichoderma* spp. M-1-D medium is more suitable for preparation of fungal culture filtrates than malt extract medium.

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