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Effect of Soil Borne Microorganisms on *Parthenium*

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ABSTRACT

Corynespora cassicola, Curvularia sp., *Curvularia lunata, Fusarium oxysporum, F. moniliforme, Monilia* sp. and *Trichoderma harzianum* were isolated from *Parthenium* infested soil of Coimbatore district of Tamil Nadu. Spore suspension as well as culture filtrates of *F. oxysporum* and *F. moniliforme* were highly pathogenic to the weed and its seed germination and their pre-emergence and post-emergence applications at 5% level resulted in complete inhibition of seed germination and 100% wilt incidence, respectively. *T. harzianum* was highly pathogenic to the *Parthenium* seeds only but not to the established weed.

INTRODUCTION

Parthenium hysterophorus L., one of the seven most dangerous weeds of the world, is an extremely prolific seed producer with an enormous seed bank estimated at 2,00,000 seeds m^{-2} in abandoned fields in India (Navie *et al.,* 1996), which remained viable in soil for more than two years (Joshi, 1991). In general, resident parasites and saprophytes present in soil play an important role in soil seed bank. Investigations were carried out to isolate microorganisms associated with *Parthenium* infested soil, study their effects on *Parthenium*, their host range and to develop commercial formulations.

MATERIALS AND METHODS

Ten soil samples were collected randomly from the cropped and non-cropped areas of Coimbatore, Tamil Nadu just below the *Parthenium* bushes alongwith leaf liters and used for isolation of microorganisms by serial dilution technique (10^{-4} dilutions) using soil extract medium. The plates were incubated for three days; fungal colonies grown in the plates were transferred immediately into agar slants; identified based on their cultural and morphological characters and further confirmed with International Mycological Institute, Kew, Surrey, England, UK.

Conidial suspension of the isolated pathogens was prepared by flooding seven days old culture on potato dextrose agar with sterile distilled water and scrapping the top of the agar surface with a sterile glass slide. Resulting slurry was strained through two layers of sterile cheese cloth and the concentration of conidial suspension was adjusted to 5×10^6 conidia per ml using a haemocytometer.

To study the effect of microorganisms on *Parthenium* germination, seeds were surface sterilized with 0.1% sodium hypochlorite for 30 sec., washed thrice with sterile distilled water, shade dried, soaked individually in spore suspension of test pathogens for 60 min and the germination test was performed by roll towel method (ISTA, 1985). Seeds soaked in sterile distilled water served as control. One hundred seeds were used for each treatment and each treatment was repeated four times. The observation on seed germination was recorded seven days after inoculation (DAI). The root and shoot lengths were also measured for 10 randomly selected healthy seedlings and vigour index was worked out as per the formula suggested by Abdul-Baki and Anderson (1973).

To study the effect of foliar spray of microorganisms, 30 days old *Parthenium* plants

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maintained in glass house (10 plants earthen pot^{-1}) were washed gently with sterile distilled water and allowed to dry. Then plants were individually sprayed in the evening hours with spore suspension+Tween 20 (0.05%) using an atomizer. Suitable control was maintained and each treatment was replicated thrice under randomized block design. The plants were then transferred into an environmental test chamber maintained at 28±2^{0}C and 95% RH immediately after spray for 48 h and then transferred to the glass house. The per cent disease index (PDI) and plant mortality were recorded 15 and 30 days after spray.

Sand maize inoculum of test pathogens was prepared and added at 5% level (w/w) to sterilized pot culture mixture (10 kg) in earthen pots to study the effect of soil application on microorganisms. The surface sterilized *Parthenium* seeds were sown at 10 seeds pot^{-1}. In another set of experiment, the sand maize inoculum of test pathogens was applied individually at 5% level to the root zone of 30 days old *Parthenium* plants maintained under glass house condition. Each treatment was replicated thrice under randomized block design. The per cent seedling emergence and wilt incidence were recorded individually for the first and second set of experiments 15 days after inoculation.

Attempts were made to assess the efficacy of talc formulation of *T. harzianum* on the germination of *Parthenium* alongwith beet root, bengal gram, ladies finger, chilli, cowpea, bajra, frenchbean, greengram, groundnut, maize, peas, pumpkin, safflower, sesame, jowar, soybean, sunflower and tomato under *in vitro* and *in vivo* conditions.

Talc formulation was prepared as per the protocol standardized by Kousalya and Jeyarajan (1988). For *in vitro* studies, talc formulation was applied at 0.125 g (2.5 kg ha^{-1}) to the germination trays containing 10 kg of fresh sterilized river sand. The surface sterilized crop seeds and *Parthenium* seeds were sown in lines at 10 seeds row^{-1}. For every five rows of crop seeds, two rows of *Parthenium* seeds were sown and the germination test was carried out as per ISTA (1985). Control was maintained without the addition of talc formulations. Each treatment was replicated four times and the germination (%) was recorded as described earlier.

A microplot experiment was carried out at orchard, Tamil Nadu Agricultural University, Coimbatore to assess the efficacy of talc formulation on seed germination and reaction of test crop plants under natural condition. Two-metre length ridges were formed at 30 cm apart. The surface sterilized crop seeds and *Parthenium* seeds were sown in individual rows at 20 seeds row^{-1} in such a way that *Parthenium* seeds (two rows) were sown for every five rows of crops. Talc formulation was applied to the soil application at 2.5 kg ha^{-1} alongwith FYM (50 kg ha^{-1}) prior to sowing and the germination (%) was recorded seven days after sowing. Each treatment was replicated four times under randomized block design.

RESULTS AND DISCUSSION

Corynespora cassicola (IMI No. 379985), *Curvularia* sp. (IMI No. 379999), *Curvularia lunata* (IMI No. 378927), *Fusarium oxysporum, F. moniliforme, Monilia* sp. and *Trichoderma harzianum* were isolated from the *Parthenium*-infested soil. Among these, *Parthenium* seeds soaked individually in the spore suspension of *F. oxysporum, F. moniliforme* and *T. harzianum* for 1 h exhibited 100% inhibition of seed germination, while others were found to exert less inhibitory effect on *Parthenium* seed germination compared with control which recorded maximum seed germination (78.7%), shoot length (5.1 cm), root length (4.3 cm) and vigour index (742.0) seven DAI (Table 1).

Spraying of *Parthenium* plants with spore suspension of *F. oxysporum* resulted in the maximum PDI and plant mortality of 74.1 and 11.7% 15 days after spray and 77.0 and 13.3%, respectively, 30 days after spray. This was followed by *F. moniliforme, Curvularia* sp., *C. cassicola, Monilia* sp. and *C. lunata.* But *T. harzianum* did

Table 1. Effect of soil microorganisms on *Parthenium* seed germination and on symptom expression

Microorganisms	Seed germination*				Symptom expression*			
					15 days after spray		30 days after spray	
	Seed germination (%)	Shoot length (cm)	Root length (cm)	Vigour index	PDI (%)	Plant mortality (%)	PDI (%)	Plant mortality (%)
Corynespora cassicola	21.0[b] (27.27)	2.0[b]	1.4[b]	74.0[b]	20.4[c] (26.85)	0.0[c] (9.10)	23.0[c] (28.63)	0.0[c] (9.10)
Curvularia lunata	21.7[b] (27.80)	2.0[b]	1.4[b]	74.0[b]	2.6[d] (5.39)	0.0[c] (9.10)	3.0[d] (5.77)	0.0[c] (9.10)
Curvularia sp.	35.2[c] (36.42)	3.4[c]	2.8[c]	218.0[c]	21.8[c] (27.87)	0.0[c] (9.10)	24.1[c] (29.38)	0.0[c] (9.10)
Fusarium oxysporum	0.0[a] (2.87)	0.0[a]	0.0[a]	0.0[a]	74.1[a] (59.39)	11.7[a] (19.89)	77.0[a] (61.36)	13.3[a] (21.34)
F. moniliforme	0.0[a] (2.87)	0.0[a]	0.0[a]	0.0[a]	60.8[b] (51.22)	10.0[b] (18.44)	63.0[b] (52.51)	11.7[b] (19.89)
Monilia sp.	43.0[d] (40.98)	4.1[d]	2.9[d]	304.0[d]	16.3[c] (23.80)	0.0[c] (9.10)	20.4[c] (26.82)	0.0[c] (9.10)
Trichoderma harzianum	0.0[a] (2.87)	0.0[a]	0.0[a]	0.0[a]	0.0[e] (5.74)	0.0[c] (9.10)	0.0[e] (5.74)	0.0[c] (9.10)
Control	78.7[e] (62.55)	5.1[e]	4.3[e]	742.0[e]	0.0[e] (5.74)	0.0[c] (9.10)	0.0[e] (5.74)	0.0[c] (9.10)

*Mean of three replications. Data in parentheses are arcsine-transformed values.
In a column, means followed by a common superscript are not significantly different at 5% level by DMRT.

Table 2. Effect of soil microorganisms on seedling emergence and wilt incidence of *Parthenium*

Treatment	15 days after inoculation*	
	Seedling emergence (%)	Wilt incidence (%)
Corynespora cassicola	19.2[c] (26.02)	11.2[bc] (19.52)
Curvularia lunata	21.7[d] (27.80)	5.00[d] (12.92)
Curvularia sp.	14.5[b] (22.37)	13.7[b] (21.70)
Fusarium oxysporum	0.0[a] (9.10)	100.0[a] (80.90)
F. moniliforme	0.0[a] (9.10)	100.0[a] (80.90)
Monilia sp.	41.2[e] (39.96)	3.7[e] (9.69)
Trichoderma harzianum	0.0[a] (9.10)	0.0[f] (9.10)
Control	78.2[f] (62.20)	0.0[f] (9.10)

*Mean of three replications. Data in parentheses are arcsine transformed values.
In a column, means followed by a common superscript are not significantly different at 5% level by DMRT.

not exhibit any symptoms on the artificially sprayed plants (Table 2). The pathogenic nature of *Curvularia* sp., *C. lunata, F. oxysporum* and *F. moniliforme* on *Parthenium* weed was already proved by Pandey *et al.* (1992) and Aneja and Manpreet Kaur (1995). In a separate study, we also observed that *C. lunata, F. oxysporum* and *F. moniliforme* were found to be associated with leaf blight disease of *Parthenium* weed.

Pre-emergence and post-emergence application of *F. oxysporum* and *F. moniliforme* individually to the soil as sand maize inoculum at 5% respectively resulted in complete inhibition of seedling emergence and cent per cent wilt incidence compared with other treatments (Table 3). Though *T. harzianum* was not pathogenic to the germinated seedlings of *Parthenium*, it caused cent per cent inhibition of seedling emergence. *T. viride* was identified as a good biocontrol agent and also pathogenic to crop plants (Farr *et al.*, 1989; Menzies, 1993).

Table 3. *In vitro* and *in vivo* studies of the talc formulation of *T. harzianum* on *Parthenium* and cultivated crops seed germination

Host tested	Variety	*In vitro* condition*		*In vivo* condition*	
		Control	Treated	Control	Treated
Beet root	Ooty 1	100.0	100.0	88.0	92.0
Bengal gram	CO 2	84.0	100.0	80.0	94.0
Ladies finger	CO 2	100.0	100.0	85.0	95.0
Blackgram	CO 4	100.0	100.0	88.0	96.0
Chilli	PMK 1	94.0	96.0	90.0	94.0
Cowpea	Paiyur 1	95.0	97.0	90.0	95.0
Bajra	CO 7	96.0	98.0	91.0	96.0
French bean	YCD 1	94.0	96.0	88.0	92.0
Greengram	VBN 1	100.0	100.0	88.0	96.0
Groundnut	VRI 2	98.0	100.0	90.0	96.0
Maize	CO 1	100.0	100.0	94.0	97.0
Peas	Alaska	55.0	60.0	46.0	50.0
Pumpkin	CO 1	82.0	90.0	70.0	82.0
Safflower	CO 1	50.0	52.0	40.0	48.0
Sesame	TMV 3	100.0	100.0	94.0	98.0
Jowar	CO 22	98.0	100.0	92.0	98.0
Soybean	ADT 1	70.0	78.0	60.0	68.0
Sunflower	CO 1	98.0	100.0	80.0	90.0
Tomato	PKM 1	100.0	100.0	88.0	92.0
Parthenium		79.0	0.0	78.0	0.0

*Mean of four replications.

None of the crop plants was susceptible to talc formulation of *T. harzianum* against seed germination of cultivated crops and *Parthenium* (Table 3). But it affected the *Parthenium* seed germination. Similarly, Kauraw *et al.* (1995) observed that the soil application of *T. viride* at 200 g m^{-2} (as soil dust or neem oil cake formulation) delayed the seed germination, shoot and root length of the weed *P. minor.*

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