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Research Note

Biocontrol of *Alternaria* leaf spot of *Vicia faba* using antagonistic fungi

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ABSTRACT: In dual culture, all the three antagonists, *viz. Trichoderma virens* Miller, Giddens and Foster, *Trichoderma harzianum* Rifai and *Trichoderma viride* Pers., ex. Fr. overgrew the colony of *Alternaria alternata* (Fries) Keissler but *T. viride* parasitized the test fungus earliest. *T. viride* exhibited highest growth rate in mono and dual culture. Studies on hyphal interaction between antagonist and test fungus revealed disorganization of protoplasmic content and lysis of host hyphae. *T. viride* exhibited 36.33 per cent disease control and proved superior in providing protection against *Alternaria* leaf spot of *Vicia faba* in field than *T. virens* and *T. harzianum*.

KEY WORDS: *Alternaria alternata*, bioagents, *Vicia faba*

Leaf spot of *Vicia faba* (Faba bean) caused by *Alternaria alternata* (Fries) Keissler is one of the most serious diseases in many areas of Bihar and Jharkhand. Under severe conditions of infection, yield loss upto 40 per cent has been reported. Due to health risk and pollution hazards by use of chemical fungicides in plant disease control, it is considered appropriate to minimize their use. Use of antagonistic fungi in this context appeared logical and safe. In this investigation, efficacy of antagonist *Trichoderma* species on *A. alternata in vitro* and in suppressing the leaf spot of *Vicia faba* under field conditions has been determined.

The studies were conducted in Department of Plant Pathology and Research Farm of Tirhut College of Agriculture, Dholi, Muzaffarpur (Rajendra Agricultural University, Bihar, Pusa) during 2000-2001. Three antagonistic fungi, *viz. Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma virens* were evaluated *in vitro* to test the antagonism against *A. alternata* by dual culture technique using potato dextrose agar (PDA) medium (Morton and Straube, 1955). Three replications were maintained for each treatment. All the plates were incubated in BOD incubator at 28 ± 1°C. Observation on colony diameter of *A. alternata* was recorded at 48, 72, 96 and 144 h of incubation.

To study the growth of antagonists and the pathogen in monoculture, 6 mm mycelial discs of antagonist and test fungus placed centrally on sterilized PDA in Petri-plate and incubated at 28 ± 1°C. Observation on colony diameter of individual organism was recorded at 24 and 48 h of incubation.

Hyphal interaction between the antagonist and the pathogen was studied when the colonies of two fungi came in contact with each other. Small

mycelial fragments from the zone of interaction of the pathogen and antagonist was taken out with the help of sterilized needle and placed on a glass slide in a drop of cotton blue, spread it out with the help of 2 needles, mounted in lactophenol and examined in an Olympus microscope for hyphal interaction. Slides were also prepared from the areas where pathogen was overgrown by the antagonist to study the hyphal interaction between the pathogen and the antagonist. Reisolation was done by taking a 6 mm disc from the area where pathogen colony had already overgrown by the antagonist and placing centrally on PDA.

Field trial was laid out in randomized block design (RBD) using a susceptible land race of *Vicia faba* in 3 replications of 3 x 1.5 m^2 plots. The bioagents *T. virens*, *T. viride* and *T. harzianum* were used as spray to test their efficacy against leaf spot. Talc preparation of *T. viride* and *T. harzianum* were used @ 4.0 g/l while *T. virens* (biomass powder) was used @ 1.0 g/l of water. The crop was artificially inoculated with the spore suspension of *A. alternata* by macerating the infected leaves @ 12 leaves/ litre of water and filtered through muslin cloth to create epiphyotic. The bioagents were sprayed during evening hours (between 4 to 5 PM) after 48 hours of artificial inoculation of the pathogen. Two consecutive sprays at an interval of 10 days were given. Final observation on per cent disease intensity was recorded 10 days after second spraying of bioagents. Per cent disease control was calculated by using the following formula and analysed statistically using RBD after angular transformation of values.

$$\text{Disease control (\%)} = \frac{C - T}{C} \times 100$$

C= Disease intensity in control plot

T= Disease intensity in treated plot

In dual culture, *A. alternata* grew freely till it reached in contact with the colony of an antagonist. At 48 h of incubation, the colony diameter of *A. alternata* in monoculture was 23.66 mm while in dual culture it ranged from 23.00 to 23.33 mm *i.e.*, on par with mono and dual culture but at 72 h, growth of the pathogen colony, in dual culture was less in comparison to monoculture due to contact and interaction with the antagonist and thereafter it ceased completely (Table 1).

The antagonists, *T. viride*, *T. harzianum* and *T. virens* overgrew the colony of *A. alternata* in 72 to 96 hours. *T. viride* colonized *A. alternata* earlier than *T. harzianum* and *T. virens*. *T. viride* produced non-volatile compound, which diffused in the medium and turned yellow. This yellowish diffusible substance might be affecting the viability of the pathogen adversely. *T. harzianum* also showed similar reactions with *A. alternata* in dual culture and produced green growth with moderate sporulation on pathogen colony. Similar trend of results with *A. alternata* in dual culture was recorded in *T. virens*. However, it produced dark green growth with abundant sporulation on pathogen colony. Similar observations on antagonism between *Trichoderma* and *Alternaria* was reported by Lal and Upadhyay (2002).

T. viride occupied 87.33 mm growth on PDA in 48 hours of incubation in comparison to 80.33 and 76.33 mm colony diameter of *T. virens* and *T. harzianum*, respectively in monoculture. *A. alternata* could attain the growth of 23.67 mm only in 48 hours (Table 1). The study indicated that all the three antagonists were faster in growth rate than the pathogen.

Microscopic observation on hyphal interactions between antagonist and *A. alternata* revealed lysis and protoplasmic disintegration of hyphae of the test fungus at many locations. Mycoparasitism through physical contact by coiling and pathogen cell lysis in case of *Sclerotium rolfsii* by *T.harzianum* has been reported by Upadhyay and Mukhopadhyay (1986). The disintegration of mycelia of test fungi may be due to action of enzymes produced by *Trichoderma* spp. (Elad *et al.*, 1982) and production of volatile and non-volatile chemical compounds (Upadhyay and Roy, 1995) and toxins (Brain and Mc Gowan, 1945). The pathogen cell lysis and protoplasmic disintegration of the mycelium by *T. harzianum* and

Table 1. *In vitro* growth of *A. alternata* and *Trichoderma* species in mono and dual culture

Antagonist	Colony diameter (mm)				
	24 h	48 h	72 h	96 h	144 h
	Monoculture				
Trichoderma virens	37.33	80.33	-	-	-
Trichoderma harzianum	35.33	76.33	-	-	-
Trichoderma viride	39.00	87.33	-	-	-
Alternaria alternata	10.00	23.67	-	-	-
SEM±	1.33	1.52	-	-	-
CD(P=0.05)	2.97	3.34	-	-	-
	Dual culture				
A. alternata + *T. virens*	-	23.00	26.33	26.33	26.33
A. alternata+*T. harzianum*	-	23.33	29.00	29.00	29.00
A. alternata+*T. viride*	-	23.33	27.33	27.33	27.33
A. alternata alone	-	23.66	35.33	44.33	66.33
SEM±	-	1.51	1.21	1.71	1.48
CD(P=0.05)		3.32	2.66	3.76	3.26

T. virens have been reported earlier (Chet *et al.*, 1981).

To test the efficacy of antagonists against *Alternaria* leaf spot of *Vicia faba* under field conditions, the bioagents were diluted in water and sprayed over the plants during evening hours. The results revealed that bioagents provided 25.93 to 36.33 per cent protection to the crop from *Alternaria* leaf spot, being maximum in *T. viride* and minimum in *T. harizanum* (Table 2). *Trichoderama* was found

Table 2. Effect of foliar spray of *Trichoderma* on the intensity of *Alternaria* leaf spot of *Vicia faba* under field conditions

Antagonist	Dose (g/ l)	Disease intensity (%)	Disease control over check (%)	Yield (q/ ha)
Trichoderma viride	4.0	42.11 (40.46)	36.33 (37.07)	10.12
Trichoderma harzianum	4.0	49.11 (44.49)	25.93 (30.48)	9.82
Trichoderma virens	1.0	47.00 (43.28)	28.93 (32.54)	10.13
Untreated control		66.11 (54.40)	-	7.13
SEM ±		0.35	0.75	0.27
CD (P = 0.05)		0.78	1.72	0.60

Figures in parentheses are angular transformed values.

to be antagonistic to *Alternaria solani in vitro* and *in vivo* and *T. koningii* gave 67.5 per cent disease control (Kumar and Singh, 1984). Yield data recorded in different biological treatments showed no significant difference (variation *i.e.*, 9.82 to 10.13 q/ ha) between them but they were significantly superior to untreated control, which yielded 7.13 q/ ha grain yield.

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