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Effect of some Antagonists on the sclerotial Germination of *Claviceps fusiformis*

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ABSTRACT

The germination of sclerotia of *Claviceps fusiformis* Loveless was significantly inhibited when placed in soil amended with *Aspergillus niger*, *Fusarium oxysporum*, *Trichoderma harzianum*, *T. viride* and *Bacillus subtilis*. The antagonists did not affect the germination of pearl millet seeds. The sclerotia colonised by the antagonists were found disintegrated and fragments of such sclerotia showed the colonies of the respective antagonists on incubation.

KEY WORDS : Pearl millet, *Claviceps fusiformis*, sclerotial germination, antagonists

Ergot of pearl millet (*Pennisetum americanum* (L.) Leeke) caused by *Claviceps fusiformis* Loveless is an important disease in India and African countries (Loveless, 1967; Ramakrishnan, 1971; Thakur and Chahal; 1987). Besides affecting crop yields, it also poses health hazards resulting from consumption of ergot-contaminated grains (Krishnamachari and Bhat, 1976).

As the disease is restricted to the spikelets, chemical sprays are not advisable because of the toxic residue problem (Prakash, 1983). Other cultural practices like adjustment of sowing date (Sharma *et al.*, 1984), burial of sclerotia (Sundaram, 1967) and pollen management (Willingale *et al.*, 1986) have been suggested to control the ergot of pearl millet. However, these methods are not practicable to farmers in the semi-arid tropics as the crop is rain-dependent. Hence, in the present investigation, biological means of controlling the disease was tested.

MATERIALS AND METHODS

Pearl millet sclerotia collected in November 1987 were stored in unsterilised sand at room temperature (18°C to 23°C) until September 1988. Twenty microorganisms were isolated from the rhizosphere and the phylloplane of pearl millet, the honeydew and the sclerotia of *C. fusiformis*. Five microorganisms *Aspergillus niger* Van Tieghem, *Fusarium oxysporum* Schl. ex Fries, *Trichoderma harzianum* Rifai, *T. viride* Pers. ex Gray and *Bacillus subtilis* (Cohn) Prazmowshi, commonly used as biocontrol agents, were selected for the present study.

In each of the 1000 ml flasks, 200 g of wheat bran moistened with distilled water (400 ml of water, 200 g of the bran) was taken. The sterilized bran was inoculated with the antagonist and incubated under room conditions (25 ± 2°C). Fifteen-day-old cultures of biocontrol agents in wheat bran were mixed with soil in pots and in field at the rate of 2% (w/w) as described by Marshall (1982). Four hundred ergot susceptible pearl millet seeds (cultivar HB-3) were sown in the pots and watered daily to check the effect of the antagonist on seed germination. To test the effect of antagonist on the sclerotial germination, four replicates of 100 sclerotia each were considered for each biocontrol agent. Four similar sets were made. The sclerotia were kept in unsterilised soil between nylon nets. First set of pots was watered daily to facilitate germination. The second, third and fourth sets of sclerotia were subjected to germination after 15, 30 and 45 days of placing in the soil. The tests were carried out at different intervals to simulate the rainfall under the field conditions. The sclerotia were observed for germination starting from seven days of watering till 30 days. The sclerotia kept in unamended soil served as control. The sclerotia kept in the field soil were also sampled once in 15 days and subjected to germination test.

The sclerotia kept in the soil were tested for the associated mycoflora. One hundred sclerotia were taken out from the soil after 45 days of sowing and mycoflora analysed by the Standard Blotter method (Anon., 1976). The sclerotia were observed under a stereo-microscope on the 50th day. The fungi present were identified and recorded. In all the experiments four replicates were maintained and the data were subjected to statistical analysis.

RESULTS AND DISCUSSION

The biocontrol agents did not affect the germination of pearl millet seeds. The germination percentages in the different antagonists ranged from 47-77 while it was 41 in the case of control but the differences were not significant. In the first set, the sclerotia in the pot soil amended with *A. niger* were completely colonized

by the antagonist and the sclerotial germination was reduced from 23.75% in control to 4%. The soil amended with other antagonists also significantly reduced the sclerotial germination when compared to the unamended soil. The inhibition in sclerotial germination was maximum in *T. harzianum* amended soil that was watered after 15 days followed by *F. oxysporum* amended soil. The sclerotia in the soil amended with *A. niger* showed a maximum of 8.5% germination. In the third set, the sclerotial germination was inhibited to the maximum in the soil amended with *T. viride* followed by *T. harzianum*. In the fourth set, further reduction was observed in the soil amended with *T. viride* and *F. oxysporum* (Table 2). In the first set, the germination of the sclerotia kept in the field soil amended with *T. viride* was inhibited to a maximum extent (84.21%) *A. niger* inhibited sclerotial germination by 71.05% over the control. *B. subtilis* maintained its inhibition capacity throughout the experimental period.

A majority of the sclerotia that were amended with the biocontrol agents were distintegrated. The pieces of sclerotia, when incubated on wet blotters, showed the presence of respective biocontrol agents. The study indicated that the sclerotia act as the substrates for the colonization of the biocontrol agents.

The antagonists used in the present study are known to be effective on many soil-borne pathogens suppressing the sclerotial germination of the pathogen. *A. niger* is effective on *Macrophomina phaseolina* (Mukhopadhyay, 1977; Pande, 1985) and *Rhizoctonia solani* (Venkatasubbiah and Safeeulla, 1984). *T. harzianum* and *T. viride* are reported to antagonise *R. solani* and *Sclerotium rolfsii* (Wiley and Kammedahl, 1981; Sivan, 1987). *B. subtilis* inhibited the growth of *R. bataticola* and *S. rolfsii* (Pande, 1985). Some attempts have also been made to control ergot pathogens by using antagonists. The development of sclerotia by *Claviceps purpurea* was affected by *Fusarium heterosporum* (Hornok and Walez, 1983). *Cerebella andropogonis* is a common inhabitant of honeydew and prevents the sclerotial development of *C. fusiformis* (Kulkarni and Moniz, 1974). *Fusarium* sp. and *F. chlamydosporum* have been reported to colonise honeydew of *C. purpurea* of rye (Mower *et al.*, 1975) and *C. fusiformis* of pearl millet (Chahal *et al.*, 1987). However, no reports are available on the prevention of the sclerotial germmination of ergot pathogens. The present study indicates that sclerotial germination of *C. fusiformis* can be effectively checked by common soil inhabiting antagonists. This inhibition of germination of sclerotia prevents the availability of ascospores of the pathogen and thus prevents primary infection of the host. The antagonists used in the present study can be used as an important management practice against *C. fusiformis*.

Table 1. Germination of sclerotia in soils amended with antagonists in pots and field

Antagonists	Germination (%) * / period of placement in soil						
	Pots				Field		
	0	15	30	45	15	30	45
Control (unamended)	23.75 [a]	10.75 [a]	9.75 [a]	6.00 [a]	9.50 [a]	5.25 [a]	3.00 [a]
Aspergillus niger	4.00 [a] (83.16)	8.50 [b] (20.98)	5.50 [ab] (43.58)	1.75 [b] (70.83)	2.75 [b] (71.05)	1.50 [b] (71.42)	1.00 [b] (66.67)
Fusarium oxysporum	5.50 [c] (76.84)	3.00 [c] (72.09)	2.75 [bcd] (71.79)	1.00 [b] (86.33)	2.00 [b] (78.94)	1.50 [bc] (71.42)	1.00 [b] (66.67)
Trichoderma harzianum	4.25 [c] (82.10)	1.50 [c] (86.04)	2.25 [bcde] (76.92)	1.25 [b] (79.00)	2.25 [b] (76.31) [b]	1.50 [bcd] (71.42)	0.75 [b] (75.00)
T. viride	7.75 [b] (67.36)	6.75 [bc] (37.20)	2.00 [c] (79.48)	1.00 [b] (83.33)	1.50 (84.21)	1.00 [bcd] (80.95)	1.25 [ab] (58.33)
Bacillus subtilis	4.75 [c] (80.00)	6.50 [cd] (39.53)	3.25 [bc] (66.66)	1.25 [b] (79.00)	1.75 [b] (81.57)	0.50 [c] (90.47)	0.50 (83.33) [b]

* = Average of 400 sclerotia

Numbers in each column followed by the same letter(s) are not significantly (P = 0.05) different according to Duncan's multiple range test

Figures in parenthesis represent percentage of inhibition over control

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