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FUSARIUM SEMITECTUM VAR. *MAJUS*—A POTENTIAL BIOCONTROL AGENT OF ERGOT (*CLAVICEPS FUSIFORMIS*) OF PEARL MILLET*

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Abstract : A *Fusarium*-like growth was invariably observed on pearl millet inflorescences infected with ergot (*Claviceps fusiformis*) both in field and greenhouse conditions. The fungus was isolated from honeydew and sclerotia, and was identified *Fusarium semitectum* var. *majus* Wollenw by IMI. Several experiments were conducted in greenhouse and field to determine parasitic potential of *F. semitectum* var. *majus* (Fsm) on ergot. Application of Fsm at various time of ergot development resulted in 83–98 per cent ovary colonization, 14–52 per cent reduced sclerotia formation, 24–43 per cent sclerotia colonization, and 46–48 per cent sclerotia disintegration over control. These positive characteristics of Fsm indicate its potential to be a useful biocontrol agent of ergot of pearl millet.

Keywords : Mycoparasitism, *Fusarium semitectum* var. *majus*, *Claviceps fusiformis*, Honeydew, Sclerotia, Pearl millet

Mycoparasitism is a potential biocontrol mechanism of fungal diseases of plants. *Fusarium sambucinum* and *Dactylium fusarioides* have been reported to parasitise *Claviceps fusiformis* Loveless, the causal agent of ergot of pearl millet [*Pennisetum americanum* (L). Leeke] (Tripathi *et al.*, 1981). Mycoparasitism of *Claviceps* spp. that cause ergot in other cereals has also been reported (Chalaud, 1940; Cunfer, 1975; Cynthia and Shattock, 1980; Futrell and Webster, 1966; Mower *et al.*, 1975; Simpson and West, 1952). Tripathi *et al.* (1981) also reported the disintegration of sclerotia in soil, and inhibition of *C. fusiformis* spore germination in association with the two mycoparasites. In the pearl millet ergot nursery at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), the growth of *Fusarium* sp. was consistently observed on ergot-infected inflorescences at honeydew and sclerotial stages both in the rainy and post-rainy seasons, but to a greater extent during the rainy season. From 1983 to 1985, attempts were made to isolate the fungus, *Fusarium* sp. and test its parasitic potential on *C. fusiformis*.

In this paper, the identity of the *Fusarium* sp., its association with *C. fusiformis* at different stages of ergot development, and its ability as a potential biocontrol agent to reduce the primary inoculum load of ergot in pearl millet fields are described.

MATERIALS AND METHODS

Isolation and identification of the fungus

Isolates of *Fusarium* were obtained from ergot infected inflorescences of pearl millet in the ICRISAT ergot nursery. Numerous samples of ergot infected florets with

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honeydew and sclerotia showing *Fusarium*-like growth were placed on potato dextrose-agar (PDA) plates and incubated at 25°C for 7 days with 12 h photoperiod. The *Fusarium* grew faster than *C. fusiformis* and thus was easily isolated by subculturing on PDA. In repeated isolations, the same *Fusarium*-like fungus was isolated. To further purify, sub-culturing was done on a *Fusarium*-selective medium, the modified Czapek dox-agar (CDA) supplemented with pentachloronitrobenzene (PCNB), malachite green and dicrysticin (Singh and Chaube, 1970). The cultural characteristics of the fungus were noted and the culture was sent to the International Mycological Institute (IMI) of Commonwealth Agriculture Bureau (CAB), Kew, Surrey, London, for further identification at the species level.

Based on the cultural and morphological characteristics [cultures pale peach to brown in reverse, aerial mycelium white and becoming somewhat compressed by the formation of effuse sporodochia which form orange pustules due to the presence of conidia in mass. Conidiophores formed in aerial mycelium have loose branching structures with formation of lateral and terminal conidiogenous cells. Conidiophores formed in sporodochia are short and compressed, and have globose basal cell (6–9 × 4–6 μ) bearing a number of short, one-celled branches which at the apex bear two to four short cylindrical to pyriform phialides which measure 5–6 × 3 μ. Conidia hyaline, orange in mass, curved fusoid with a wedge-shaped basal cell, 3–7 septate. 3–5 septate 35–45 × 4–5 μ; 6–7 septate 40–55 × 4–6.5 μ. Chlamydospores, often sparse, are intercalary both in the mycelium and the conidia. They are globose, smooth, 8–10 μ daim. (Gerlach and Nirenberg, 1982)], the fungus was identified as *Fusarium semitectum* var. *majus* Wollenw., Z. ParasitKde 3 : 325, 1931 by IMI.

Ergot inoculation

Aqueous ergot conidial suspensions (ca. 1×10^6 conidia/ml) obtained from previously inoculated inflorescences of pearl millet in greenhouse were used in all the experiments. Plants were bagged at the boot leaf stage and inoculated at protogyny ($\geqslant$ 75 per cent stigma emergence) by spraying conidial suspension by briefly removing bags (Thakur *et al.*, 1982). Ergot severity scorings were taken using a standard ergot severity assessment key (Thakur and Williams, 1980). The time course of flowering events and development of ergot is illustrated in Fig. 1.

Unless otherwise mentioned, all field experiments were done at the ICRISAT ergot nursery where high relative humidity was maintained by operating overhead sprinkler irrigation twice a day for 1 hour each during noon and evening.

Association of *Fusarium* with ergot

Colonization of ovary : In a greenhouse, ergot-infected plants of a pearl millet male sterile line 5141A were sprayed with aqueous spore suspension (1×10^6 spores/ml) of *Fusarium semitectum* var. *majus* (Fsm) grown on PDA for 7 days at 25°C. Five plants were sprayed each at 24, 96 and 144 h after honeydew appearance. Water sprayed and unsprayed plants served as control.

Five florets were sampled randomly from each plant 2 days after Fsm-spray and were dissected to obtain ergot-infected ovaries. The ovaries were transferred onto CDA plates and examined for Fsm colonization 3 days after incubation at 25°C with 12 h photoperiod.

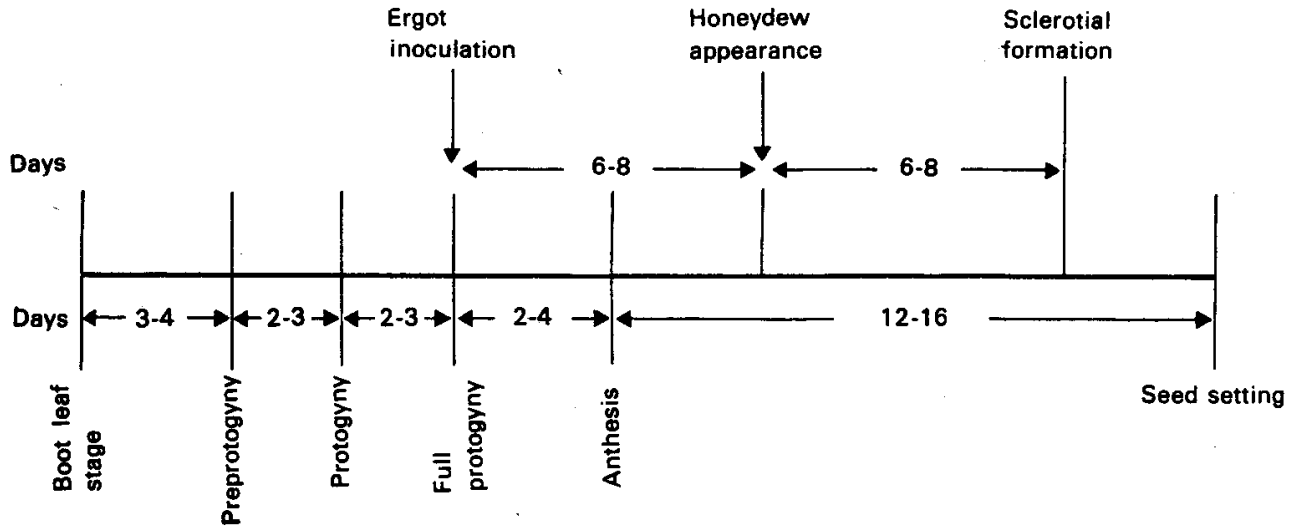

Fig. 1 : A generalized scheme of time course of flowering events in relation to ergot inoculation and development in pearl millet (Preprotogyny = emergence of panicle from the boot; Protogyny = emergence of stigmas; Full protogyny = ⩾ 75 per cent stigma emergence).

Colonization of sclerotia by Fsm : In a field experiment during the 1983-84 post-rainy season, three ergot susceptible pearl millet hybrids, BJ 104, ICH 206, BK 560, were subjected to the following treatments: Fsm at preprotogyny + ergot at protogyny; both Fsm and ergot (1 : 1) at protogyny; Fsm at honeydew appearance, Fsm at sclerotial appearance; Fsm at protogyny; and ergot at protogyny. The experiment was conducted in a randomized block design with 3 replications and 10 plants per replicate. To determine the extent of sclerotial colonization by Fsm, sclerotia collected from the above treatments were divided into two equal lots. One lot (30 sclerotia/treatment) was surface sterilized with 0.1 per cent $HgCl_2$ for 3 min, washed thoroughly with sterile, distilled water, and then cut into longitudinal halves. These were placed on modified CDA with cut-surface in contact with the medium. The other lot (not surface sterilized and not cut) were planted similarly on CDA. The plates were incubated for 6 days at 25°C with 12 h photoperiod before observation for colonized and non-colonized sclerotia.

Effect of spore concentration of Fsm on ergot development

Aqueous spore suspensions of five concentrations (1×10^4 to 5×10^6 spores/ml) prepared from 7-day old growth of Fsm on PDA at 25°C were sprayed on ergot inoculated inflorescences of BJ 104 at the honeydew stage and rebagged immediately. The experiment was conducted in a randomized block design with four replications and 10 inflorescenes for replicate. Observations were made on sclerotial formation and sclerotial colonization by Fsm.

Effect of time of Fsm spray on ergot development

In a field experiment in ergot nursery, plants of BJ 104 and Togo 42-10 were subjected to the following treatments: Fsm at honeydew appearance; Fsm + ergot (1 : 1) at protogyny; Fsm alone at protogyny; and ergot alone at protogyny. The experiment was conducted in a randomized block design with four replications and 20 plants per replicate. Observations were recorded 20 days later on ergot severity, sclerotial formation and sclerotial colonization by Fsm.

Effect of Fsm-colonization on compactness and disintegration of sclerotia

One sample of sclerotia collected from different treatments of the above experiment was tested for their compactness and disintegration. Compactness was tested by measuring hardness of sclerotium using a 'Seed Hardness Tester'. The pressure required to break sclerotia was measured in kg/cm^2. Samples of 80 sclerotia were used for each treatment.

The other sample of 80 sclerotia from each treatment were placed in double layer muslin cloth bags (20 sclerotia/bag) and buried in potted soil 3 cm deep in a greenhouse. The pots were watered to keep the soil moist. Bags were removed 30 days later and evaluated for their disintegration by simply pressing individual sclerotia between the right hand-thumb and forefinger. Percent disintegrated sclerotia was noted for each treatment.

RESULTS

Colonization of ergot-infected ovaries and sclerotia by Fsm

Spraying of ergot-infected inflorescences with Fsm to 24, 96 and 144 h after honeydew appearance resulted in heavy colonization (83–98 per cent) of ovaries (Table 1). The unsprayed and water sprayed inflorescences produced 0 to 33 per cent ovary colonization. Both surface sterilized and unsterilized sclerotia from different treatments showed colonization by Fsm. The surface sterilized sclerotia showed 2 to 12 per cent internal colonization (Fig. 2). The non-surface sterilized sclerotia showed 6 to 51 per cent colonization. The maximum colonization of sclerotia (51 per cent) occurred when a mixture of Fsm and ergot (1 : 1) was sprayed at protogyny and this was significantly higher ($P \leqslant 0.5$) than other treatments.

Effect of Fsm spore concentrations on sclerotial development and sclerotial colonization

Significant differences were noted for per cent sclerotia formation and per cent sclerotia colonization in different concentrations. The maximum reduction in sclerotia formation (52 per cent of control) and maximum sclerotial colonization (18 per cent) occurred at the highest spore concentration (5×10^6 spores/ml) (Table 2). There was an increasing levels of sclerotial colonization with increasing Fsm spore concentration.

Effect of time of Fsm spray on ergot development, sclerotial colonization, compactness and disintegration

Mean ergot severities in BJ 104 and Togo 42–10 were in the range of 63–92 per cent in different treatments of Fsm with ergot (Table 3). Ergot severity was not much influenced by Fsm. However, there were significant reductions in sclerotia formation due to presence of Fsm. There were 45 and 40 per cent reduced sclerotial formation over control in BJ 104 and Togo 42–10, respectively when a mixture of Fsm and ergot (1 : 1) was sprayed at protogyny. The maximum colonization of sclerotia, 43 per cent in BJ 104 and 24 per cent in Togo 42–10, also occurred in the mixture treatment, and these were significantly higher than other treatments.

TABLE 1 : Per cent colonization of ergot-infected ovaries of a pearl millet male sterile line 5141A sprayed with *Fusarium semitectum* var. *majus* (Fsm) at different times of honeydew development in greenhouse

Treatments	Fsm spray time (h) after honeydew appearance		
	24	96	144
Fsm spray	94	98	83
Unsprayed	3	33	31
Water spray	0	13	18

Based on 33-56 ovaries placed on CDA medium at 25°C.

TABLE 2 : Effect of *Fusarium semitectum* var. *majus* (Fsm) spore concentrations on sclerotial formation and sclerotial colonization in a pearl millet hybrid BJ 104 under field conditions, rainy season 1985

Fsm spore concentration (spores/ml)*	Sclerotia formation (per cent)	Reduction in sclerotia formation (per cent) over control	Sclerotia colonization (per cent)
0	56	—	0
1×10^4	48	14	4
1×10^5	27	52	6
5×10^5	42	25	5
1×10^6	31	45	6
5×10^6	27	52	18
SE	± 3.2		± 2.2

*Fsm spore suspensions were sprayed at honeydew appearance and 40 plants were used in 4 replicates for each concentration.

Sclerotia formed in Fsm sprayed treatments were less compact than those formed in unsprayed treatment. Based on hardness test pressures required to break Fsm-sprayed sclerotia were 1.68 to 2.05 kg/cm^2 compared to 2.18 to 2.43 kg/cm^2 for sclerotia in control treatment. Sclerotial disintegrations in field soil from Fsm spray treatments were 46 to 48 per cent more than in control (Table 3).

DISCUSSION

This is the first report of mycoparasitism of *C. fusiformis*, the pearl millet ergot pathogen, by *F. semitectum* var. *majus*. Previously reported mycoparasites of pearl millet ergot include *Cerebella andropogonis* Ces., that prevented sclerotial development (Kulkarni and Moiniz, 1974), *Fusarium sambucinum*, and *Dactylium fusarioides* (Tripathi *et al.*, 1981).

At ICRISAT Center, *F. semitectum* var. *majus* naturally colonizes ergot-infected florets of pearl millet. The high relative humidity required for ergot development also favours the development of *F. semitectum* var. *majus*, and the high concentration of soluble sugars in the honeydew of ergot appears to support its growth. In these studies

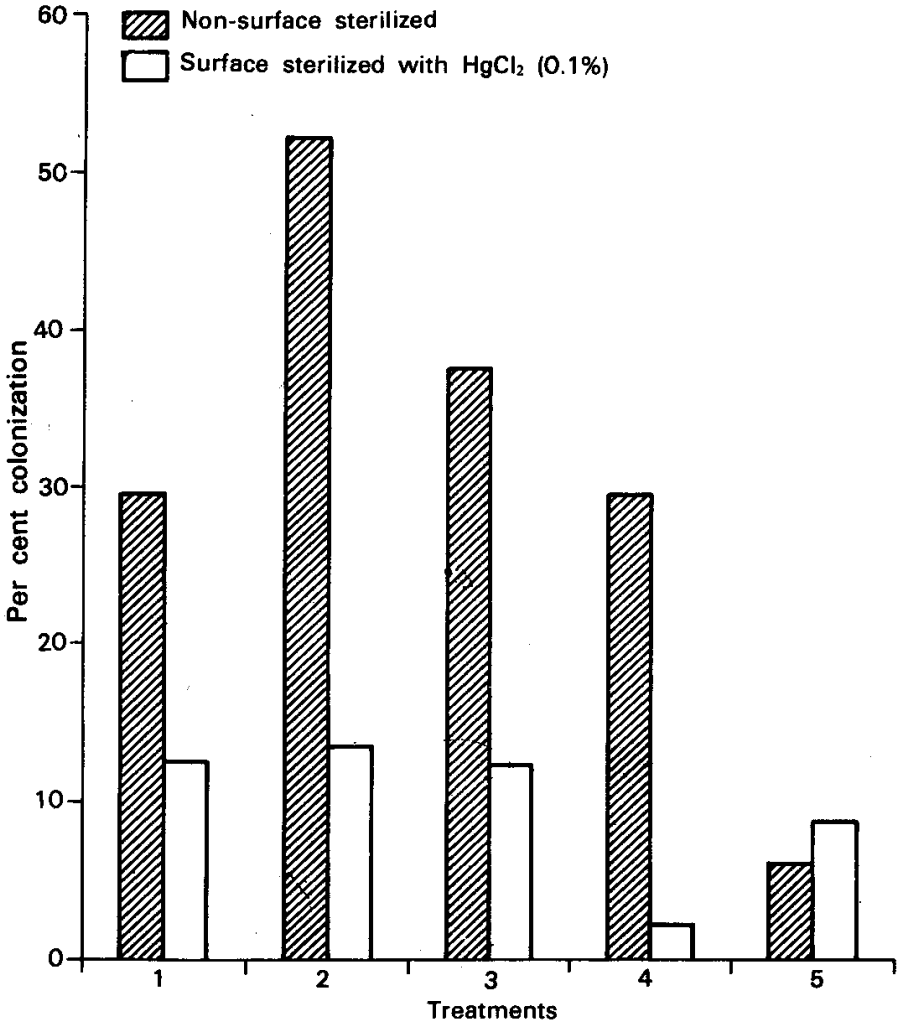

Fig. 2 : External (non-surface sterilized) and internal (surface sterilized with $HgCl_2$ 0.1 per cent) colonization by *Fusarium semitectum* var. *majus* (Fsm) of *Claviceps fusiformis* (Cf) sclerotia obtained from the following inoculation treatments: 1 = Fsm at preprotogyny + Cf at protogyny; 2 = Fsm + Cf at protogyny; 3 = Fsm at honeydew initiation; 4 = Fsm at sclerotial development; 5 = Control (only Cf at protogyny)

F. semitectum var. *majus* reduced sclerotial number and enhanced disintegration of sclerotia in soil, although it did not inhibit or reduce ergot infection. These characteristics suggest that *F. semitectum* var. *majus* is capable of acting as an effective biocontrol agent of pearl millet ergot. It would, however, be important to know its inhibitory effects on germination of *C. fusiformis* spores, and the mechanisms involved in preventing sclerotia formation and enhance sclerotial disintegration in soil. In the case of *F. sambucinum*, it has been shown that culture filtrates contain a heat-labile factor which inhibits germination of *C. fusiformis* spores (Tripathi *et al.*, 1981).

Mower *et al.* (1975) showed in experiments that *F. roseum* 'Sambucinum', a mycoparasite on *C. purpurea*, is a highly effective biocontrol agent for ergot of wheat. *F. heterosporum*, a mycoparasite which grows on *C. purpurea* honeydew on rye and barley, reduces sclerotial formation but does not penetrate ergot conidia or hyphae (Cunfer, 1976). But there have been no reports of the commercial use of any of these mycoparasites to control ergot in wheat, barley or rye. This is probably because ergot is not so important as in these crops.

To be a successful biological control agent of ergot, a mycoparasite should inhibit or reduce germination of conidia, sclerotia, and ascospores and disintegrate sclerotia. Rapid disintegration of sclerotia could effectively reduce the sclerotial population in the soil, which constitutes the primary source of inoculum for the succeeding crop. *F. semitectum* var. *majus* can be easily cultured and rapidly multiplied in large quantities for field use, but the practical application of it, as a possible biocontrol agent for commercial use in the field, will require further testing. Our future research will attempt to study selection of a more virulent strain of Fsm, mechanism of sclerotial disintegration and its interaction with *C. fusiformis*.

TABLE 3 : Effect of *Fusarium semitectum* var. *majus* (Fsm) and/or ergot inoculation at different flowering times on ergot development and sclerotial formation, colonization, compactness (hardness) and disintegration of sclerotia in two pearl millet genotypes, rainy season 1985

Treatments	Ergot severity* (per cent)		Sclerotia formation* (per cent)		Sclerotia colonization* (per cent)		Sclerotia hardness† (kg/cm²)		Sclerotia disintegration (per cent)
	BJ 104	Togo 42-10	BJ 104	Togo 42-10	BJ 104	Togo 42-10	BJ 104	Togo 42-10	BJ 104
Fsm + ergot (1 : 1) at protogyny	87	63	33	29	43	24	1.9	1.9	77.5
Fsm at honeydew appearance	90	70	54	48	6	7	1.7	2.1	76.3
Ergot alone at protogyny	92	71	60	49	0	5	2.2	2.4	52.5
Fsm alone at protogyny	14	1	6	1	17	3	—	—	—
Water at protogyny	1	1	1	1	0	<1	—	—	—
SE	±12	±1.7	±1.9	±1.9	±2.3	±1.5	±0.09	±0.09	±6.76

*Mean of 80 inflorescences from 4 replications.
†Based on 80 sclerotia from 4 replications.

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