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Pathogenicity, morphology and identification of *Claviceps fusiformis* incited ergot disease of pearl millet

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Abstract
The present study was on pathogenicity, morphology and identification of *Claviceps fusiformis* which responsible for ergot disease of pearl millet and observed most favorable stage of panicle emerged at which highest disease was observed. The disease was found to be very severe in all pearl millet growing areas causing heavy destruction. The main aim of this study clearly demonstrated that *C. fusiformis* is a primary pathogen in pearl millet, causing ergot disease. Isolates of *C. fusiformis* were collected from ergot infected panicle (at honey dew stage) of pearl millet in the fields around Jobner, Jaipur. The pathogen was isolated from sample on PDA media in Petri plate and make a mass multiplication for pathogenicity test. Inoculated the conidial suspension on healty plant and symptoms test morphology of pathogen are identified according to Thakur and King 1988 and observed that highest PDI (53.1 per cent) at 75 per cent panicle emerged followed by 42.6 per cent PDI at 50 per cent panicle emerged than 35.88 per cent PDI at 25 per cent panicle emerged.

Keywords: *Claviceps fusiformis*, ergot, honey dew stage, identification

Introduction
Pearl millet (*Pennisetum glaucum* (L.) R.Br.) is the extensively drought tolerant warm season cereal grown on 27 million ha in some of the harsh, dry-semi-arid and arid tropical environments of sub-Saharan Africa and South Asia. In India third after rice and wheat grown area under pearl millet, among cereals. It is more tolerant to high temperatures than any other cereal. The optimum temperature for the germination of bajra seed is from 23 to 32°C. 500-800 mm rainfall is optimum for pearl millet production. It is a highly cross-pollinated species, with outcrossing rates of more than 85 per cent, because of its protogynous nature of flowering. This stage is favorable for ergot incidence. It uses mainly for cattle feed, poultry, starch and alcohol industry. It is noted that demand for bajra from the health-conscious food products industry is increasing as it contains more fibre and is good for heart and diabetic patients. Pearl millet is also an important fodder crop in the summer season, as most of the other crops cannot withstand hot temperatures. However, there are some biotic and abiotic constraints in cultivation of bajra, such as ergot, downy mildew, smut, shoot fly, stem borer, drought, extreme moisture and heat moisture stress. (Reddy *et al.*, 2013) [19]

Pearl millet production is concentrated in Rajasthan, Maharashtra and Gujarat which account for 70 per cent of production in India. Bajra is usually grown as a dryland dual purpose grain and fodder crop although it is sometimes irrigated in India, particularly the summer crop grown mainly as a forage crop (Basavaraj *et al.*, 2010) [4].

Today, ergot is one of the principal factors preventing the realization of the high grain yield potential of bajra hybrids in Africa and Asia. In addition, it reduce the quality of the grain in an infected crop by contaminating it with the alkaloid containing sclerotia of ergot. (Bhinde and Sheth 1957, Patel *et al.*, 1958, Ramkrishnan 1971, Bhat, Roy and Tulpule 1976, Krishnamachari and Bhat 1976) [6, 15, 5]. Ergot, caused by *C. fusiformis* loveless, was a minor disease of pearl millet (*Pennisetum americanum* (L.) Leeke) in India until the early 1970s when it became a serious problem in crops of the recently developed and adopted commercial F1 hybrids (Arya and Kumar 1976, Ramkrishnan 1971 and Thakur and Williams 1980) [23].

Panicles are bagged at the boot leaf stage, using selfing bags of parchment paper, and inoculated at the full protogyny stage (>75% stigma emergence) with an aqueous honey dew conidial suspension (approximately 10^6 conidia ml^{-1}) of *C. fusiformis*.

The suspension was obtained from the honey dew of previously inoculated panicles of a susceptible cultivar. High relative humidity (>80%) was maintained during flowering and early grain filling by sprinkler irrigation, provided twice daily on rain free days. The bags were removed 20 days after inoculation, and panicles were scored for ergot severity based on the percentage of florets infected (Thakur and Williams 1980) [23].

C. fusiformis has been described as the main ergot pathogen of pearl millet (*Pennisetum typhoideum*, now *P. glaucum*) in Africa and India (Loveless 1967, Thakur *et al.*, 1984) [13, 24]. The oldest specimen of *C. fusiformis* came from Ghana in 1925 (Loveless, 1967) [13]. In Africa, *C. fusiformis* was the typical ergot parasite of *Pennisetum* and *Cenchrus* (Loveless 1964a, 1967) [14, 13].

Studies at ICRISAT (Hyderabad) during 1979, revealed that in case of *C. fusiformis* both macro and micro conidia from honeydew germinated producing a germ tube at either end. The germ tube extended became setate and again formed macro or micro conidia.

Ramkrishnan (1963) [18] observed that the conidia of *C. fusiformis* retained their viability upto 13 month, indicating that the fungus could survive in nature in conidial form. Siddiqui and khan (1973) [20] suggested that *C. fusiformis* tertiary conidia were perhaps infective propagules.

C. fusiformis produces two types of conidia: macroconidia and microconidia, both in culture and in honeydew on infected pearl millet panicles. Macroconidia hyaline, fusiform, unicellular, 12.0-26.5x2.5-6.0 µm, producing 1-3 germ tubes from their ends or sides. Microconidia hyaline, globular, unicellular, 2.5-11.0x1.0-5.0µm, producing only one germ tube. Both macroconidia and microconidia are able to produce additional microconidia in chains from conidiophores formed by the germ tubes (Prakash *et al.*, 1983) [17]. Sclerotia elongate to globose, 3.6-6.1 x 1.3-1.6 mm, pale to dark brown, with or without conidia-containing cavities Germination by 1-16 fleshy purplish stipes, 6-26 mm long, each bearing apical globular capitulum, light to dark brown with numerous perithecial ostioles (Thakur *et al.*, 1984) [24].

Asci interspersed with paraphyses, emerge through ostioles. Ascospores hyaline, filiform, non-septate, 103-176 x 0.5 µm (Thakur *et al.*,1984) [24].

Pazoutova *et al.* (2008) [16] isolated of *Claviceps* species with lunate to fusiformis macroconidia collected from panicoid grasses in texas and Zimbambe and described as new species based on anamorphs since no teleomorphs were available.

Isolates of the pearl millet ergot pathogen *C. fusiformis* were collected from eight locations in India and studied for morphological and pathogenic variations. Sclerotia were examined for shape, size and cavities, and residual macro-and microconidia for their size and viability. Sclerotia from Aurangabad and ICRISAT Centre were the largest (5× 2.5 mm) and heaviest (1.4g/100 sclerotia), and from Mysore the smallest (3 × 2 mm) and lightest (0.4 g/100 sclerotia). The average number of cavities (furrows) was minimum in the Mysore collection (4/sclerotium) and maximum in the Kovilpatti collection (15/sclerotium). Large variations occurred in size and ratios between the number of macro- and microconidia residual on sclerotia from different locations. Time to initiation of germination of macroconidia from sclerotia in sterile distilled water at 25 °C varied from 24 hours in Aurangabad and Jobner collections to 50 hours in the Kovilpatti collection. The macroconidia of the Pune collection did not germinate. The isolates differed in growth characteristics on Kirchoff's agar at 25°C. In a pathogenicity test using 10-day-old culture inoculum in six pearl millet genotypes (3 resistant and 3 susceptible), some isolates were more virulent than others. (Chahal *et al.*, 1985) [8].

Conidia of *C. fusiformis* from Africa and India were long (mean 18.9 µm), mostly straight and fusiform. (Pazoutova *et al.*, 2008) [16].

The results obtained on morphological characteristics conidial size of the isolate included macroconidia hyline, fusiform, unicellular, 12.0-26.5× 2.5-6.0 µm. Microconidia hyline, globular, unicellular 2.5-11.0×1.0-5.0 µm in length and width. (Bhosale 2019) [7].

Colonies of *Claviceps* on T2 medium, (14days, 24°C) 21-48 mm in diameter; diffuse and markedly radiating margin, colony mostly velutinous, consisting of highly sporulating conidiophores giving a powdery appearance, raised with typically cerebriform wrinkles in the centre and plane toward the margin, obverse off-white to grayish, reverse similar or light brown getting typically reddish brown in age in some strains, soluble diffuse pigment yellowish to reddish brown to vinaceous. Macroconidia: fusiform, straight, rarely lunate (10-28 × 3-9 µm, mean 19 × 5 µm). Loveless (1967) [13], Thakur *et al*.(1984) [24].

Sclerotia of African and Indian isolates of *C. fusiformis* from pearl millet contain agro-clavine and elymoclavine as the main alkaloid components; minor or trace components are chanoclavine, setoclavine, penniclavine and, occasionally, festuclavine. Both also readily produce clavines in submerged culture *in vitro* (Banks *et al.*, 1974, Bhat *et al.*, 1976, Singh and Husain 1977, Kumar and Arya 1978) [3, 5, 21, 12].

Material and Method

The infected samples of Pearl millet showing symptoms of ergot (honey dew) disease were collected from the farmer's fields around Jaipur, brought to the laboratory, isolated honey dew drops with the help of sterilized inoculated loop and dry it. Dried drop of honey dew washed thoroughly with distilled water, dried drop of honey dew cut with sharp sterilized with 0.1% aqueous solution of mercuric chloride ($HgCl_2$) for two minutes and then washed by giving three changes with sterile distilled water to remove traces of mercuric chloride and blot dried. These surface sterilized and dried drops of honey dew were then inoculated on the autoclaved, solidified and cooled PDA (Potato dextrose agar) medium in Petri plates under aseptic conditions of laminar air flow cabinet. Inoculated plates were then incubated in BOD incubator at 28±1ºC temperature. After week incubation, the well developed mycelial growth of the test pathogen, free from any contaminant was obtained. Following hyphal tip technique, test pathogen was transferred aseptically on the PDA slants. Through frequent sub-culturing, the pathogen was purified and its pure culture maintained on agar slant and stored in refrigerator for further studies.

Proving the pathogenicity

Pathogenicity test was carried out in pot grown pearl millet plant at cage house of department of plant pathology of SKNCOA, Jobner Jaipur. The pathogenicity was test with four treatments and five replications. Panicles were covered with paper bags, as and when emerged from boot leaves, bagging was carried out to avoid external inoculums. The plant was inoculated by conidial suspension (1×10^6

conidia/ml) by hand held atomizer, at three different stage of plants 25 per cent panicle emerged, 50 per cent panicle emerged, 75 per cent panicle emerged and un-inoculated plant. The inoculated panicles were covered with paper bags to maintain high relative humidity inside and to avoid the external contamination. Inoculated plants were kept in plastic cover cage house and kept for incubation at 25ºC with more than 80% relative humidity with bags enclosing the panicles. Further observations were made for development of the disease. Disease severity was recorded on a standard 0-8 disease rating scale (0 to 8 scales of Thakur and king 1988). (Table 1)

Table 1: Ergot disease severity rating scale. (Thakur and King 1988)

Rating scale	Symptoms and lesions	Disease reaction
0	No ergot infected florets in the panicle	highly resistant
1.	1% ergot infected florets in the panicle	
2.	> 1-5% ergot infected florets in the panicle	
3.	> 5-10% ergot infected florets in the panicle	resistant
4.	> 10-20% ergot infected florets in the panicle	Susceptible
5.	> 20-35% ergot infected florets in the panicle	highly susceptible
6.	> 35-40% ergot infected florets in the panicle	
7.	> 50-75% ergot infected florets in the panicle	
8.	100% ergot infected florets in the panicle	

Result

Pathogenicity test

Typical symptoms of honey dew and sclerotial development on head of artificially inoculated pearl millet plant were observed that 75 per cent panicle emergence (53.1 per cent PDI) followed by 50 per cent panicle emergence (42.6 per cent PDI) (Table 2 Fig. 1 Plate 1 Plate 2). Likewise, minimum disease severity was observed at 25 per cent panicle emergence (35.88 per cent PDI).

Reisolation

The fungus was reisolated from each treatment of panicle and were compared with original culture of the test pathogen. The same was found identical to that of original culture, thereby confirming the test of pathogenicity.

Table 2: Pathogenicity test of *Claviceps fusiformis* on pearl millet at different growth stage of panicle under controlled conditions

S. No.	Treatment	**Per cent Diseases Index***
1	Inoculation at 25% panicle emerged	35.88 (36.77)
2	Inoculation at 50% panicle emerged	42.6 (40.72)
3	Inoculation at 75% panicle emerged	53.1 (46.75)
4	Un-inoculated	0.00
	SE(m) +	0.36
	C.D. at 5%	1.09
	C.V.	2.60

* Average of five replication

Figures in parentheses are angular transformed values

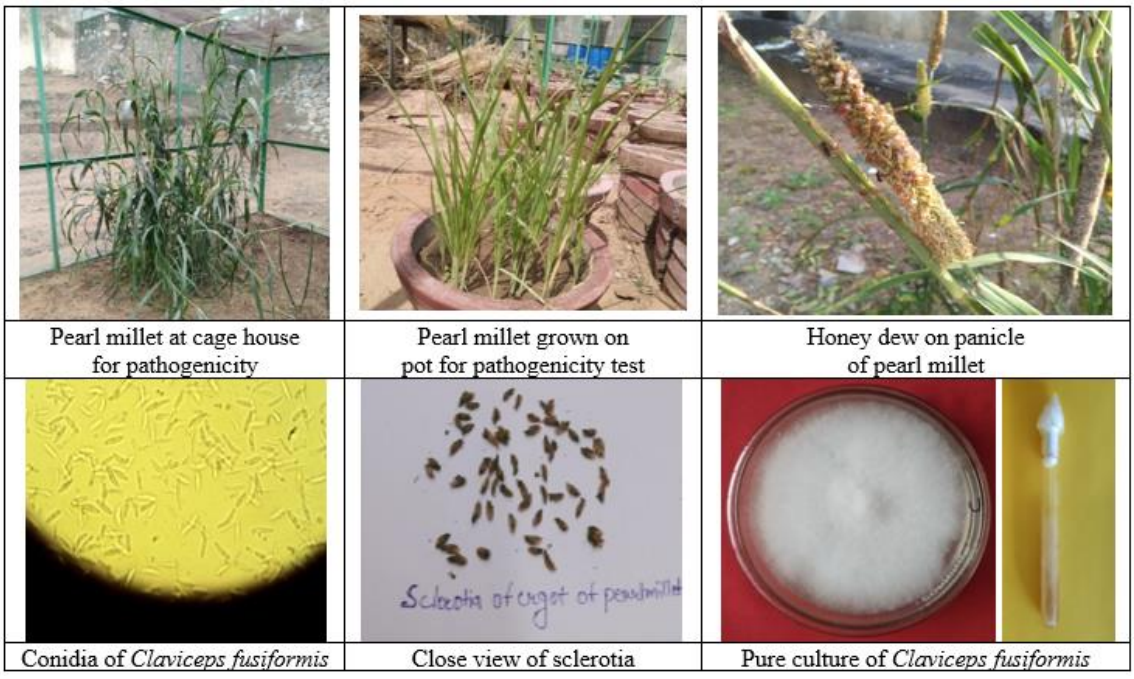

Plate 1: Pathogenicity of *Claviceps fusiformis* incited ergot disease of pearl millet.

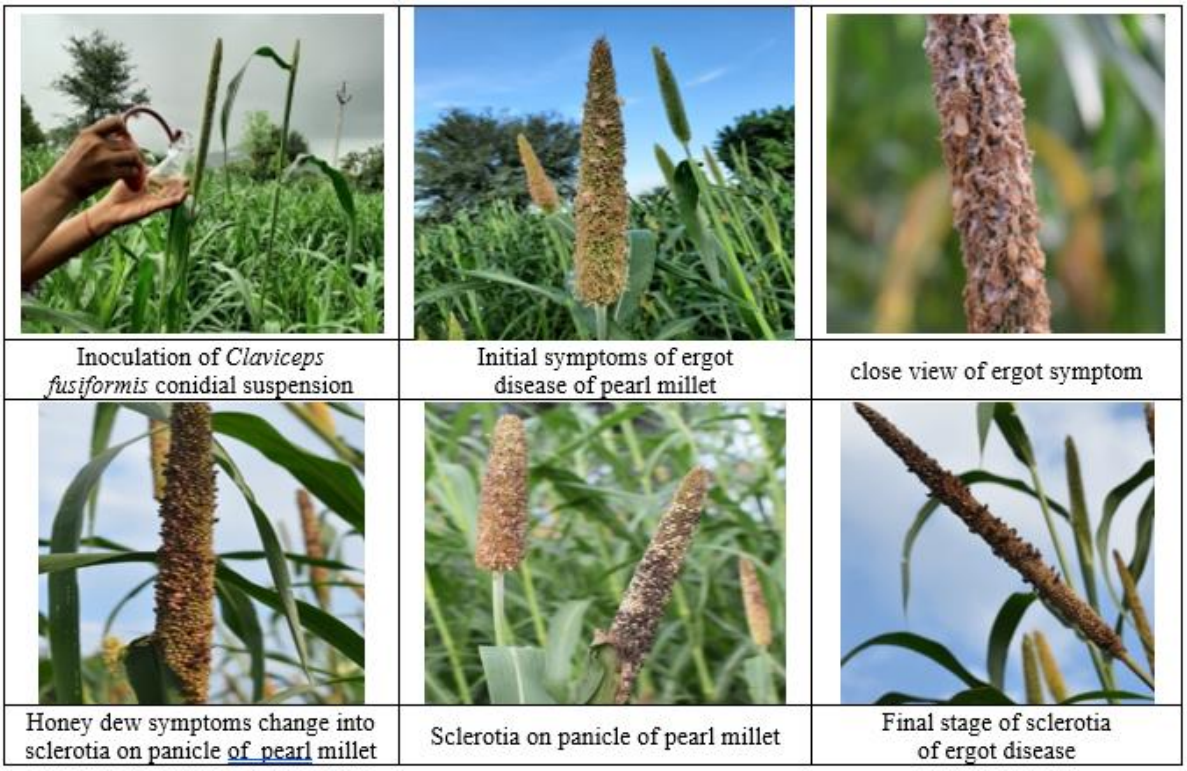

Plate 2: Symptoms cycle of ergot disease of pearl millet

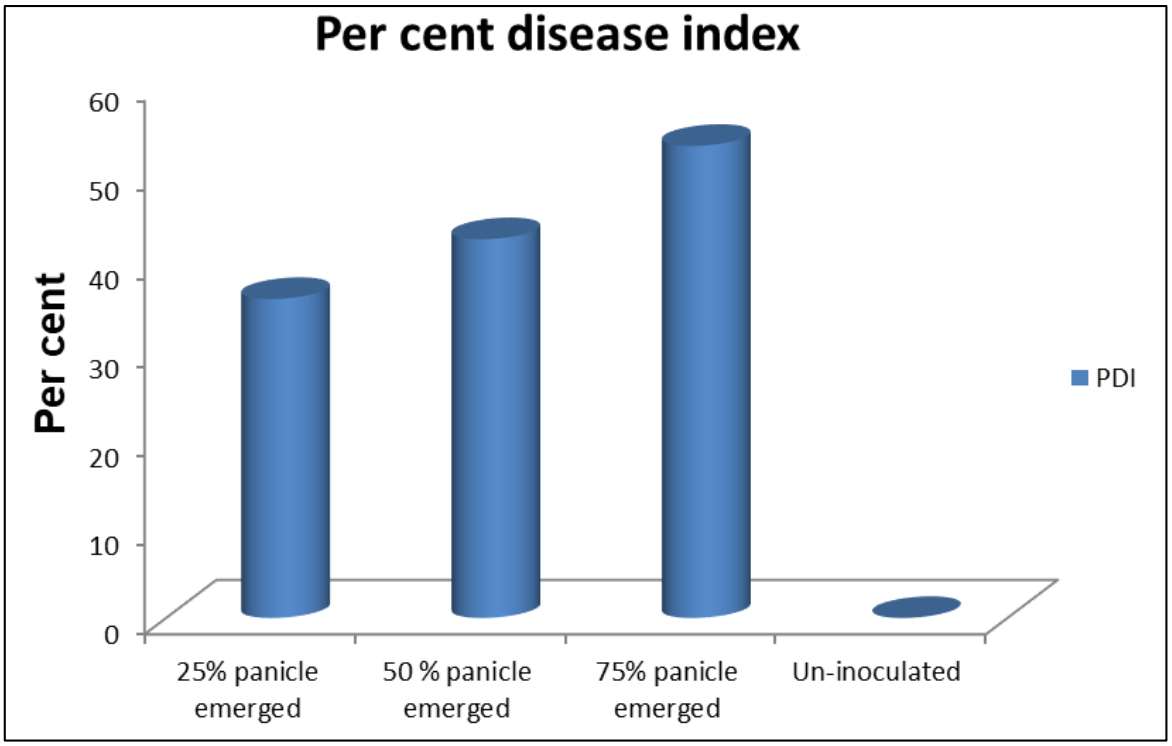

Fig 1: Effect of stage of panicle emergence on development of ergot disease of pearl millet

Discussion

The fungus collected from ergot infected panicle of pearl millet from different locations was isolated on culture (PDA) under aseptic condition in laboratory. The fungus was purified by using "hyphal tip cut technique" after isolation of fungus, PDA plates were kept in BOD at controlled conditions and stored in refrigerator for use in further studies. The pathogen formed uniformly dense colonies on potato dextrose agar. A whitish mycelia growth appeared on PDA which later turned into brownish black when culture was old and formed sclerotia. After 10-15 days, fungus developed black hard sclerotia in the peripheral area of the colony.

The fungus was identified as *Claviceps fusiformis* based on unique morphological and cultural characteristics formation was seen. Dark brown to black structures with a pointed apex 'sclerotia' was observed. The maximum disease severity was observed in 75 per cent panicle emergence with 53.1 per cent PDI. The observation of present studies are found similar with

the reports of the earlier worker where Frederickson *et al.* (1996) [9] reported sphacelial (conidial) 'honey dew' sporulation on Pearl Millet.

Bandyopadhyay *et al.* (1998) [2] observed that ergot only attacks unfertilized ovaries. A few or all ovaries within florets on a panicle are individually infected, specifically in male sterile lines or hybrids with fertility resortation problems. There were two obvious symptoms of infection in the field. The first and most obvious was the production of sugary fluid (honey dew) from infected florets. The second was the presence of fungal sphacelia or sclerotia between the lemma and palea of infected florets.

Fredericksen and Ovody (2000) [10] observed that honey dew was thin or viscous, sweet and very sticky. With time, honey dew can become uniformly yellow-brown in colour. It may remain as intact droplets, or drip on to uninfected florets, seeds, leaves and ground. Honey dew contains the infectious conidia (primarily macrocondia) and these conidia germinate on the surface and produce secondary conidium which has a white colouration. The fungal sphacelia or sclerotia of *C. Africana* are not very noticeable prior to production of honey dew. However, the sphacelium may or may not develop in place of seed even before honeydew is produced.

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