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Integrated management of Sorghum ergot Disease caused by *C. sorghi*

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ABSTRACT: Ergot can irreparably damage sorghum nurseries and genetic stock. Disease-related damages in South Africa and India are 10–80%. This pathogen causes severe yield loss in hybrid seed production of sorghum. This study was planned to have disease management practices, by studying the characteristics of pathogen, through biological agents. *C. sorghi*'s sphacelia or sclerotia were barely visible in honeydew. Physical characteristics helped identify *C. sorghi*. Mycelium forms micro, macro, and secondary conidia. Microconidia were hyaline aseptate and 2.5–5 m in diameter. Macro conidia were hyaline, aseptate, rectangular to oval, 13133.7 to 14127.5 m dia, and constricted in the centre. *C. sorghi* (MW281790) was identified as the disease's causal agent. Seven fungicides were tested *in vitro* at 0.0.05, 0.075, and 0.1% against *C. sorghi*. Mycelial growth was inhibited most by carboxin 37.5% + thiram 37.5% WS at 0.05%, followed by tebuconazole 25.9% EC (0.1%) (90.63%). *Bacillus subtilis* (EPC 5), one of six bioagents studied in vitro employing dual culture, inhibited mycelial growth by 56.87%. In 2019–20, a pot culture was started after the *in vitro* investigation. Spraying 25.9% EC (0.1%) tebuconazole reduced incidence to 9.99% and increased grain yield to 2009 kg/ha compared to 1278 kg/ha for the control. Spraying tebuconazole 25.9% EC (0.1%) @ 1ml/l resulted in a minimum incidence of 13.34% and a benefit–cost ratio of 1.71 in a 2020–2021 field investigation.

Keywords: Ergot, sclerotia, bioagents, fungicides.

INTRODUCTION

Ergot (Sugary disease) is a limiting factor in hybrid seed output, especially if seed set in male sterile lines is delayed due to non-synchronous flowering. Spikelets are vulnerable to ergot infection due to fast seed production in disease-prone conditions. Ergot harms sorghum nurseries and breeding material. India and South Africa have 10-80% illness losses (Bandyopadhyay *et al.,* 1996). *Sorghum bicolor* (L.) Moench is a food crop in semi-arid and sub-arid regions. Sorghum may grow on soil unsuitable for most cereals (Rosewich, 1996). It's eaten, fed, drunk, and fodder. India has 8.71 million tonnes of sorghum. Tamil Nadu produces 612 kg/ha on 4.01 lakh hectares. 4.6 lakh tonnes generated (www.apeda.gov.in). Tamil Nadu, Karnataka, Maharashtra, Andhra Pradesh, Uttar Pradesh, Gujarat, and Rajasthan are sorghum-growing areas. Hybrids with high yields have expanded sorghum output over the years. Sorghum, a difficult crop, faces abiotic and biotic stresses. At different growth phases, crops are vulnerable to fungal, bacterial, and viral diseases. Sorghum production is hampered by downy mildew, leaf blight, smuts, rust, anthracnose, charcoal rot, grain mould (complex aetiology), ergot or sweet sickness, and stripe disease (a tenui virus transmitted by *Perigrinus maidis*). "Sugary disease" is caused by *C. sorghi*. By colonising floret ovaries with fungal tissue, ergot hinders seed production (Fredericksen and Odvody 2000). This threatens hybrids and hybrid seeds, especially in male sterile lines with weak nicking or delayed seed set. In India, where hybrids are cultivated and seed corporations are situated, 10-80% seed production losses have been observed due to ergot (Sangitaro *et al.,* 1997; Fernando *et al.,* 2004). The *Claviceps* fungus lives in grass and rye ovaries (as well as other related plants). It's the species' major attribute. Important genus sclerotia species include *Claviceps purpurea*, *Claviceps fusiformis*, *Claviceps paspali*, and *Claviceps africana*. Ergot fungi *C. sorghi* and *C. cynodontis*. It produces alkaloids on grasses. Claviceps produced approximately 80 mycotoxins that caused cytotoxicity and alkaloid accumulation in human primary cells, according to Schiff, (2006); Mulac and Humpf (2011). Since millet is a significant crop, disease management is required as a pesticide substitute (2019; Manzar *et al.,* 2021). Increased geographic prevalence and impact of the disease in countries dependent on hybrid seeds have boosted the urgency for

ergot research. Since, farmers are familiar with fungicide usage, management of residue free and combined with biological agents will be investigated (Gueye *et al*., 2020).

MATERIALS AND METHODS

A. Isolation and identification of ergot pathogen

Small sections of the ergot-infected panicle components were cut up and surface sterilised in 1% sodium hypochlorite for 3 minutes before being washed five times with distilled sterile water. The sphacelia was grown on PDA and T2 agar media and incubated at 25°C with a 12-hour light cycle for 30 days. To comprehend the pathogenic variability of the fungus, the isolate was described. Based on visual and molecular analysis, the fungal was identified.

The following observations were made in order to describe the culture:

- Radial growth (cm)
- Colony type: compact/ compact -cottony
- Culture color
- Puckering nature: Low/ Moderate / High
- Sporulation: Present / Absent.

Using the C-TAB method, *Claviceps* sp. was molecularly confirmed. It was amplified using the primers ITS1 and ITS4. Using the software primer express v2.0, PCR primers were made using specific nucleotide sequences found in the tubulin intron 3 region (Tooley *et al.*, 2002), which can distinguish the Claviceps species used in this experiment.

B. Pathogenicity test

Sorghum plants that were grown in pots were used for the pathogenicity test. Paper bags were used to cover panicles as they emerged from boot leaves in order to prevent the spread of external inoculums. When the plants were in the flowering stage and the spikelets had new stigmas, panicles were injected with mycelium cultivated in plates by spraying. When the stigmas of the 50% spikelet developed, the inoculum was sprayed using a hand sprayer. Paper bags were placed over the inoculated panicles to maintain a high relative humidity within and prevent external contamination. The inoculated plants were housed in a glass house and incubated at a temperature of 25°C with a relative humidity of more than 80% while having bags covering the panicles.

C. In vitro evaluation of systemic fungicides against C. sorghi

The effectiveness of seven fungicides against the pathogen was examined in a lab setting at three different concentrations. The outcomes are shown in Table 1

The effectiveness of the fungicides described above was evaluated using the poison food technique. Prior to doing the experiment, the pathogen *C. sorghi* was cultured on PDA medium in Petri plates for fifteen days. On the basis of the active ingredient and whole product present in the chemical, fungicide suspension was made in PDA by adding the necessary quantity of fungicide to achieve the appropriate concentration. Each of the disinfected petri plates received 20 ml of a poisoned medium. A 0.5 cm mycelial disc was cut from the periphery of a ten-day-old culture, placed in the centre, and incubated at 25°–20°C until the fungus' development reached the control plate's periphery. Three replications were maintained for each treatment, and suitable checks were also kept without the addition of any fungicide. The colony's diameter was measured twice, and the average value was calculated. It was calculated what percentage of growth was inhibited. Using Vincent's approach, it was possible to calculate the percentage of growth inhibition (1947).

I = 100 (C-T) / C Where, I = Per cent inhibition of mycelium C = Growth of mycelium in control T = Growth of mycelium in treatment.

D. In vitro evaluation of bio agents

On *C. sorghi*, six bioagents were evaluated. Below is a list of the antagonists utilized in the study along with their source.

PDA in the amount of 20 ml was added to sterilised petriplates and let to solidify from previously raised juvenile cultures of both host pathogens and fungal bio agents By providing enough space between the two discs, a 0.5 cm fungal disc containing the test fungus and the appropriate bio agents was concurrently transferred aseptically to petriplates. To assess bacterial bioagents, fungus mycelial discs were retained at opposite ends, with bacteria streaked in the middle. For each treatment, three replications were kept. The Petri dishes were kept at PDA in the amount of 20 ml was added to sterilised petriplates and let to solidify. By leaving enough space between the two discs from previously established young cultures of both the fungal bio agents and the host pathogen, 0.5 cm fungal discs of the test fungus and the appropriate bio agents were transferred aseptically to petriplates simultaneously. To assess bacterial bioagents, fungus mycelial discs were retained at opposite ends, with bacteria streaked in the middle. For each treatment, three replications were kept. The petriplates were kept at a constant temperature of 25 $\pm 10^{o}$C until the colony reached the edge of the control plate. The test fungus and bio agents' colonies' diameters were measured, and the percentage of inhibition was computed. Statistics were used to analyse the data.

E. Evaluation of bio agents and fungicides under pot culture experiment

In a pot culture investigation, the potent bioagents and fungicides were tested against *C. sorghi*. When the plants were in the flowering stage and the spikelets had fresh stigmas, sorghum panicles were sprayed with sclerotia for inoculation. The inoculum was sprayed using a sprayer as soon as the stigmas of the 50% spikelet appeared. The treatments were started five days following the sclerotia injection. Following the previously stated 0–9 scale, disease incidence and

severity were recorded. Furthermore, a statistical analysis of the % disease index (severity) was performed. For statistical analysis, average values were taken into consideration. In order to perform a statistical analysis, an arc sine transformation was made because the data were recorded as percentages. After reaching physiological maturity, the yield per pot was measured and expressed in kg/ha. Statistics were used to assess the yield data.

Recording of ergot incidence

Disease incidence and severity will be recorded by following 0-9 scale.

$$\text{Percent incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Recording of ergot severity. The ergot severity was measured using a 0–9 scale created by Mayee and Datar (1986). In the current study, the severity of ergot was measured by observing all the panicles of sorghum plants. The scale's specifics are shown below.

Scale Percent area infection. 0 No incidence;1 Up to 1% spikelet's infected; 3 1-5 % spikelet's infected; 5 5-10% spikelet's infected; 7 11-20%spikelets infected; 9 Above 20% spikelet's infected. Further, these scales were converted into severity (Percent Disease Index PDI) using formula given by Wheeler (1969).

$$\text{Percent Disease Index} = \frac{\text{Sum of individual ratings}}{\text{Number of observation} \times \text{maximum grade}} \times 100$$

For statistical analysis, average values were taken into consideration. After reaching physiological maturity, yield per plot will be measured and expressed in kg/ha. After the seeds are properly dried, the weight of 1000 seeds will be recorded. A statistical analysis of the yield and 1000 seed weight data is required.

F. Efficacy of bioagents and fungicides, under field condition against C. sorghi

In the sick plot area, sowing was moved to a new location with CSH 30 sorghum. In the lab, *C. sorghi* sclerotia and broth were collected for spraying in the field to cause the symptom. When the plants were in the flowering stage and the spikelets had fresh stigmas, the sorghum panicles CSH 30 were sprayed with broth culture for inoculation. The inoculum was sprayed using a sprayer as soon as the stigmas of the 50% spikelet appeared. The cannabis culture study's effective therapies were taken for field research. The treatments, which included foliar spraying of *Bacillus subtilis* (EPC 5), *Bacillus* sp., tebuconazole 25.9% EC, hexaconazole 5%EC, carboxin 37.5%WP + thiram 37.5% WP, propiconazole 25% EC, and mancozeb 75%WP, were administered twice, with a 15-day gap between each treatment.

RESULTS

A. Identification of Pathogen C. sorghi

After 12 days of inoculation, a pure culture of the fungus was produced, showing initial whitish growth that eventually turned light yellow to pink with a slight puckering tendency. In order to do further research, this pure culture was subcultured once again in T2 agar and PDA slants. The fungal colony began as a white, cottony growth with abundant aerial mycelium in culture and progressively changed to light yellow, then pink with some puckering. Conidia were formed by mycelium in three different sizes: micro, macro, and secondary. Hyaline aseptate, 2.5–5 m in diameter, spherical to sub-spherical, the microconidia were. Hyaline, aseptate, rectangular to oval, 13133.7 to 14127.5 m dia in size, and slightly constricted at the centre, were the characteristics of macroconidia. Hyaline, aseptate, pear-shaped secondary conidia were 11.3–18.8, 5-7.5 m in size.

B. Pathogenicity

After 10 days following vaccination, the panicles started to show signs of infection. On the infected panicle ovaries of the artificially inoculated plants, honey dew was later observed in addition to the typical signs like white sphacelium on the ovaries of panicles. The symptoms are shown here after being photographed. Re-isolating the pathogen from these afflicted ovaries allowed researchers to compare its morphological characteristics to those of the pathogen's original culture, which was identical in every way. Consequently, it was determined that *C. sorghi* was the disease's causative agent (MW281790).

C. In vitro efficacy of fungicides

Seven fungicides were tested in vitro against *C. sorghi* at concentrations of 0.0.05, 0.075, and 0.1%.

Table 1: *In vitro* study efficacy of fungicide against *C. sorghi.*

	Concentration		
Fungicides	**0.05**	**0.075**	**0.1**
Hexaconazole 5%EC	87.13 (68.98)	89.88 (71.45)	93.56 (75.30)
Tebuconazole 25.9% EC (0.1%)	90.63 (72.18)	95.00 (77.08)	98.67 (83.38)
Propiconazole 25%EC	85.00 (67.21)	88.13 (69.85)	91.23 (72.77)
Mancozeb 75%WP	68.63 (55.94)	78.50 (62.38)	83.50 (66.03)
Captan 75%+hexaconazole 5%	59.75 (50.62)	65.88 (54.26)	72.13 (58.13)
Hexaconazole 4%EC+Zineb 68% WP	88.13 (69.85)	89.75 (71.33)	92.84 (74.48)
Carboxin 37.5%+Thiram 37.5% WS	92.38 (73.98)	95.63 (77.93)	98.23 (82.35)
Control	00.00 (0.00)	00.00 (0.00)	00.00 (0.00)
SEd	3.72	3.09	2.51
CD (0.05)	7.89	6.55	5.32

Arc sine transformation is used for values in parenthesis.

The carboxin 37.5% + thiram 37.5% WS at 0.05% concentration showed the greatest mycelial growth suppression (92.38%), followed by tebuconazole 25.9% EC (0.1%) (90.63%), hexaconazole 4%EC + zineb 68% WP (88.13%), and hexaconazole 5%EC (87.13%), two equally effective treatments. The combination of captan 75% + hexaconazole 5% at a concentration of 0.05% caused the least inhibition to be seen (59.75%). carboxin 37.5% + thiram 37.5% WS had the highest mycelial growth inhibition at 0.075% concentration (95.63%), followed by tebuconazole 5.36W/W (95.00%), hexaconazole 5%EC (89.88%), and captan 75% + hexaconazole 5%, which had the lowest inhibition at 65.88%. At 0.1% concentration, carboxin 37.5% + thiram 37.5% WS had the highest percent inhibition (98.23%), followed by tebuconazole 5.36W (98.67%), hexaconazole 5%EC, and captan 75% + hexaconazole 5% had the lowest percent inhibition (72.13%) (table 1).

D. In vitro efficacy of fungicides

Maximum inhibition was obtained for *Bacillus subtilis* (EPC 5) at a level of 56.87, followed by *Bacillus* sp. isolate 4 at a level of 50.00, and it was not statistically different from control (Table 2).

Table 2: *Invitro* study Efficacy of antagonist against *C. sorghi.*

S. No.	Name of the antagonists	Percent Inhibition over control
1.	*Bacillus* sp. 1	26.38 (30.90)
2.	*Bacillus* sp. 2	46.87 (43.21)
3.	*Bacillus* sp. 3	34.50 (35.97)
4.	*Bacillus* sp. 4	50.00 (45.00)
5.	*Bacillus subtilis* (EPC 5)	56.87 (48.95)
6.	Control	00.00 (0.00)
	SEd	1.68
	CD (0.05)	3.61
	CD (0.01)	5.01

Arc sine transformation is used for values in parenthesis.

E. Pot culture study

As a result of either competition (overgrowing) or antibiosis, the bioagents dramatically slowed the growth of *C. sorghi* (exhibiting inhibition zones). In comparison to all other bioagents examined, *Bacillus subtilis* (EPC 5) showed the greatest reduction in *C. sorghi* colony growth (56.87%) followed by *Bacillus* sp. 4 (50.00%) (Table 3).

Table 3: Efficacy of fungicide and antagonists against *C. sorghi* under pot culture experiment.

S. No.	Treatments	Incidence	PDI	Percent inhibition over control	Yield (kg/ha)
1.	*Bacillus subtilis* (EPC 5)	27.78 (31.81)	22.23 (28.13)	57.74	1411
2.	*Bacillus* sp. strain 4	34.45 (35.94)	29.97 (33.19)	50.00	1368
3.	Tebuconazole 25.9% EC (0.1%)	9.99 (18.43)	6.67 (14.97)	73.30	2009
4.	Hexaconazole 5%EC (0.1%)	13.34 (21.42)	10.00 (18.43)	69.97	1884
5.	Carboxin 37.5%+Thiram 37.5% WS (0.1%)	12.23 (20.47)	8.89 (17.35)	71.08	1992
6.	Propiconazole 25%EC (0.1%)	19.99 (26.56)	15.51 (23.19)	64.46	1652
7.	Mancozeb 75%WP (0.1%)	22.23 (28.13)	18.89 (25.76)	61.08	1601
8.	Control	75.57 (60.38)	79.97 (63.41)	0.00	1278
	SEd	1.99	0.85		
	CD (0.05)	4.2	1.81		
	CD (0.01)	9.07	2.49		

Values in parenthesis are arc sine transformed.

Tebuconazole 25.9% EC (0.1%) spraying produced a minimal incidence of 9.99%; it was then closely followed by sprayings of carboxin 37.5% + thiram 37.5% WS (0.1%) @ 1ml/L 12.23% and hexaconazole 5%EC (0.1%) @ 1ml/L 13.34. With the untreated control, a maximum incidence of 75.57% was observed. The lowest severity of 8.89 PDI with spraying of carboxin 37.5% + thiram 37.5% WS (0.1%) followed by 6.67 PDI with spraying of tebuconazole 25.9% EC (0.1%) and both of these severity levels are on par with one another. Significantly, untreated control had maximum severity of 79.97 PDI. With more tebuconazole 25.9% EC (0.1%) sprays, grain production was increased. Sprays were shown to have the maximum grain yield in 2009 kg/ha, followed by carboxin 37.5% + thiram 37.5% WS (0.1%) sprays (1992 kg/ha). These two numbers were comparable to one another and much greater than those of other spray treatments. Significantly, the control treatment had the lowest grain yield (1278 kg/ha).

F. Field evaluation

Table 4: Efficacy of bioagents and fungicides for the management of *Claviceps* sp. under field condition in 2020-21.

S.No.	Treatments	PDI	Percent reduction over control	Yield (kg/ha)	BC ratio
1.	*Bacillus subtilis* (EPC 5)	29.90 (33.15)	38.83	1998	1.19
2.	*Bacillus* sp.	32.23 (34.59)	34.06	2009	1.22
3.	Tebuconazole 25.9% EC (0.1%)	10.23 (18.65)	79.07	2094	1.50
4.	Hexaconazole 5%EC (0.1%)	12.23 (20.47)	74.98	2094	1.85
5.	Carboxin 37.5%WP+Thiram 37% WP (0.2%)	11.12 (19.48)	77.25	2083	1.40
6.	Propiconazole 25%EC (0.2%)	18.89 (25.76)	61.35	2035	1.33
7.	Mancozeb 75%WP (0.2%)	19.90 (26.49)	59.28	2029	1.45
8.	Control	48.88 (44.36)		1398	1.12
	SEd	1.32		94.14	
	CD (0.05)	2.60		201.93	

Figure in parenthesis represents value of arc sine transformation.

Tebuconazole 25.9% EC @ 1 ml/l spraying resulted in a minimal disease incidence of 10.23%, which was followed by carboxin + thiram (0.2%) with a disease incidence of 11.12%, which was comparable to the disease incidence of 12.23% by hexaconazole 5% EC @ 1 ml/l. With untreated control, a substantial maximal incidence of 48.88% was noted. Tebuconazole 25.9% EC (0.1%) and hexaconazole 5% (0.1%) had the maximum grain yield of 2094 kg/ha followed by carboxin 37.5%+thiram 37.5% WS (0.1%) sprays with 2083 kg/ha. These three were comparable to one another and much better than other spraying treatments. Significantly, the control treatment had the lowest grain yield of 1398 kg/ha. The chemical with the highest benefit-cost ratio was hexaconazole 5%EC (0.1%) (1.85) (Table 4).

DISCUSSION

Sorghum is a significant cereal crop that is farmed as a food crop in semi-arid tropics and sub-arid regions all over the world. It ranks fourth in importance behind rice, wheat, and maize. Sorghum is a drought-resistant crop that adapts well to a variety of ecological circumstances and can grow in soils that are unsuitable for most other cereals while still producing respectable yields (Rosewich, 1996; Ruparao and Gadi, 2019). According to Reis *et al.* (1996), ergot is a significant endemic disease that has recently spread to Central and South America. It poses a threat to sorghum production not only in India but also in America and Australia (Bandyopadhyay *et al.* 1998). When climatic conditions are conducive to infections at blooming, ergot can also cause extensive damage to male viable cultivars in farmer's fields (Molefe 1975; Sangitaro and Bade, 1975; Kukedia *et al.,* 1987). The authors noticed a similar condition in the Vangoor administrative zone of the Mahbubnagar district in Andhra Pradesh, India, in October 1999. During the rainy season of 2000, the disease expanded to the other 13 administrative zones in the district (Navi *et al.,* 2001).

In India, *C. sorghi's* sorghum ergot was first detected in 1915. Later, in 1924, the illness was identified in Kenya. It is currently extensively available in South Africa, East Africa, and Western Africa. When hybrid seed manufacturing started in South Africa in the 1960s, the pathogen turned into an economic issue. Before 1991, when Frederickson, Mantle, and de Milliano determined that *C. africana* was the pathogen responsible for ergot in Africa, it was thought that *C. sorghi* was the causal agent. South America, South East Asia, Australia, India, Southern and Eastern Africa, *C. africana*, *C. sorghi*, and *C. sorghicola*, as well as South America, South East Asia, Australia, and India, are all major populations of Claviceps (Bandyopadhyay *et al.,* 1996).

By artificially inoculating sorghum panicles with the pathogen's conidia during the crop's flowering stage, the pathogenicity of the fungus was demonstrated. Various approaches are used to demonstrate an organism's pathogenicity. Spore/conidial suspension is the most efficient inoculation technique, according to Mc Laren *et al.* (1992). In the current investigation, spray inoculation using the conidial suspension approach was used, and this led to the emergence of symptoms.

Tebuconazole and hexaconazole, two non-systemic fungicides that were examined, demonstrated the greatest degree of inhibition of all the non-systemic fungicides tested (0.075%, 0.1%, and 0.2%). At all concentrations, propiconazole and mancozeb were the least effective. Similar patterns were also seen in *C. sorghi*, according to Hegde (1998), who noted that

Mancozeb, a non-systemic fungicide, suppressed mycelial development only at concentrations of 0.3%. (Prom and Isakeit 2003).

According to the research results of the dual culture technique, *Bacillus subtilis* and *Bacillus* sp. isolate 4 showed the strongest antagonistic relationships. When compared to bacterial antagonists, all species of *Bacillus* shown greater mycelial inhibition. This is explained by *Trichoderma* spp greater its capacity for competition. Over 278 volatile chemicals, including alkanes, alcohols, ketones, pyrones (lactones), furanes, monoterpenes, and sesquiterpenes, were found in *T. harzianum* by Mostafa *et al*. (2011) and Siddique *et al*. (2012; Sudha *et al*. (2019; 2021; 2022).*T. asperellum* mVOCs increased the expression of defense-related genes and chemicals in *A. thaliana*, according to Kottb *et al*. (2015). Nonanol was generated by *Bacillus amyloliquefaciens* VOCs from Canola, which inhibits sclerotia and ascospore germination (2004).Kong *et al*.(2020) discovered that 3-methyl-1-butanol from *Rahnella aquatilis* reduces conidia formation in pine rhizosphere soil. The potential biocontrol agents against *C. africana* are examined by Bhuiyan *et al.* (2003) *in vitro*.

CONCLUSION

Even though there haven't been many studies on the pathogen and treatment of the disease, there isn't much data available on biological and chemical management studies or disease severity. Therefore, it is necessary to thoroughly research each of these factors in order to advise farmers on an efficient management approach and the fundamentals of the disease.

FUTURE SCOPE

Biological control agents as bioformulation can be employed with mVOCs as a biocontrol agent for alkaloid management in sorghum sustainable agricultural production. Future research should investigate any residual toxicity or hazards associated with bioagents / fungicides in animal feedings

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