Garlic as a Biocontrol Agent for Sorghum Ergot

S.D. Singh and S.S. Navi

Genetic Resources and Enhancement Program–Crop Health Division, International Crops Research Institute for the Semi–Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India

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Abstract

Ergot (*Claviceps sorghi*) is a serious limiting factor to the production of sorghum (*Sorghum bicolor*), specially when its flowering coincides with rains and low temperature (20–22°C). In an attempt to control this disease, we tested several biocontrol agents during 1991 to 1993 at ICRISAT Patancheru. Crude garlic extract was found most effective. The extract gave complete inhibition of conidial germination in concentration of up to 9 per cent. Prophylactic spray containing 12 per cent crude garlic extract gave 98–100 per cent control under greenhouse conditions. Under field conditions, the spray gave about 90 per cent ergot control during the post–rainy seasons (February–March) or during the non–rainy days of rainy season. During the rainy season, the sprays were less effective. The extract in concentration of more than 12 per cent was toxic. However, the extract was non–toxic to pollen germination in a concentration of upto 12 per cent. Being non–toxic to humans, the extract can be effectively used as an environmentally–friendly method of control of this disease. However the economy and the feasibility of its use are to be determined.

Key words: Garlic, sorghum ergot, environmentally–friendly control

ज्वार *(सोरघम बाइकलर)* के उत्पादन में अर्गट *(क्लीवीसेप्स सोरघी)* एक गंभीर सीमित कारक है, मुख्यत: जब पुष्पकता के समय वर्षा व कम तापक्रम (20-22° से.) हो। इस बीमारी के नियंत्रण के लिए प्रयास किया गया। इकरीसेट, पटनचेरी में हमने 1991 से 1993 तक कई जैविक नियंत्रकों की जाँच की। लहसुन का निचोड़ मुख्य प्रभावी पाया गया। निचोड़ की 9 प्रतिशत सान्द्रता ने कोनिडिया के अंकुरण को पूर्णतया संदमित किया। लहसुन के निचोड़ की 12 प्रतिशत सान्द्रता ने ग्रीनहाउस परिस्थिति में 98-100 प्रतिशत रोग नियंत्रण किया। परिक्षेत्र परिस्थिति में, वर्षा के तहत (फ्ररवरी-मार्च) के बाद छिड़काव से 10 प्रतिशत अर्गट नियंत्रित किया गया। वर्षा ऋतु काल में छिड़काव कम प्रभावी रहा। 12 प्रतिशत से अधिक निचोड़ की सान्द्रता विषैली थी। जबकि, 12 प्रतिशत सान्द्रता तक निचोड़ पराग अंकुरण के लिए निष्प्रभावी था। मानव वर्ग के लिए विषहीन रहा। निचोड़ को इस बीमारी के नियन्त्रण के लिए पर्यावरण मित्र के रूप में उपयोग में लिया जा सकता है। तथापि, इसका आर्थिक प्रभाव और उपादेयता दोनों ही अभी ज्ञात करना है।

Ergot [*Claviceps sorghi* (Kulkarni, Seshadri and Hegde)] of sorghum [*Sorghum bicolor* (L.) Moench] is a widely distributed disease in Africa and Asia (CABI, 1987). It is a serious limiting factor in hybrid seed crop grown during the cooler months (October–January) in India (Sundaram, 1980). The disease is particularly devastating if flowering in male sterile and restorer lines is asynchronous and it coincides with rains. Substantial losses can occur in male fertile cultivars also in farmers' fields (Bandyopadhyay *et al.*, 1996). Several methods have been applied to control ergot in the past years. Cultural practices like adjustment of planting dates, (Sangit Rao *et al.*, 1979, Anahosur and Patil, 1982), sowing pathogen free seed, removal of collateral hosts in and around the fields, and rouging of infected plants (Sundaram, 1968) were found ineffective. Use of fungicides is impractical, uneconomic (Anahosur, 1979), and may be associated with toxicity problems. Resistant cultivars are not yet available. Therefore, the disease continues to be a major problem.

Because of the growing concern about the fungicide associated–toxicity and environmental pollution, there is an increasing demand for safe and environmentally–friendly disease control methods. At ICRISAT Patancheru, several such methods were attempted to control sorghum ergot during 1991 to 1993. These include application of mycoparasites (*Fusarium* spp) and spraying sorghum panicles with crude extracts of neem (*Azadirachta inidca* Linn,), Sadahdhatura, (*Datura alba* Nees), onion (*Allium cepa* Linn.), and garlic (*A. sativum* Linn). This paper reports results of the

application of crude garlic extract for the control of sorghum ergot.

Materials and Methods

Garlic extract. Stock garlic extract was prepared by crushing 500 g cleaned garlic cloves in 500 ml water. Non–crushed fibrous tissues were filtered out through muslin cloth. The resultant was considered 100 per cent concentration. The extract was diluted with water in the ratio of 1:1 (50%), 1:2 (33%), 1:3 (20%), 1:5 (16%), 1:7 (12%), 1:9 (9%) and 1:12 (8.33%) (v/v).

Production and maintenance of inoculum. Initial inoculum was produced by spraying ground sclerotial (perfect stage of ergot) powder suspended in water on a susceptible cultivar, bulk Y. Later, the inoculum was multiplied and maintained on bulk Y by spraying honey–dew (imperfect stage of ergot) suspension. The honeydew stage of the pathogen was maintained in a field from August to March and during the rest of the year on potted plants in a greenhouse.

Inoculation. Freshly collected honeydew was suspended in distilled sterile water (DSW). A conidial concentration of 1 x 10^6 conidia ml^{-1} was maintained for all inoculations. Spray inoculation was done when stigmas just began to appear at the tip of the panicles. Controls were maintained by spraying DSW. Inoculation was done at about 4 pm. All the inoculated and control panicles were bagged with parchment paper bags. To maintain high relative humidity necessary for ergot infection, bagged panicles were sprayed with water three–time–a–day in all greenhouse experiments. In field, artificial humidity was not created.

In vitro studies

Effect of garlic extract on conidial germination. A 10–µl conidial suspension of *C. sorghi,* taken with a sterile inoculation loop (CCP Scientific, Inc.), was thoroughly mixed with 50 µl garlic extract on clean glass slides. The slides were incubated at 25°C in moist chambers (prepared by lining both lids of petriplates with moist blotting papers) for 20 h. Controls were maintained by mixing 10 µl conidial suspension in 50 µl DSW. Each treatment was replicated on two slides for all six concentrations. The experiment was repeated three times.

Effect of garlic extract on pollen grain germination. Viable pollen grain of cultivar bulk Y were collected early in the morning (6 am) and were uniformly dusted (using settling tower technique) on garlic extract smeared on 2 per cent water agar that was solidified on microscopic slides. The slides were incubated in moist chambers at 25°C. Each treatment had three replications. Germination was counted after 10 min. A pollen grain was counted as germinated if its germtube length was greater than its diameter. About 15–20 pollen grains were observed for germination in each of the 10 randomly selected microscopic field (40X) in each replication. Germination was studied in three, crude garlic extract concentrations (20, 16 and 12 per cent), only. The experiment was repeated thrice.

Greenhouse studies

Effect of prophylactic spray in greenhouse. Ergot susceptible cultivar bulk Y, was grown in 20–cm diameter plastic pots. Each pot had five plants. At flowering, florets of all the panicles from five potted plants were emasculated. Twelve pots with emasculated florets were grouped into three sets each with four pots. In each set, four pots containing unemasculated panicles were also included. Four pots from each set (2 with emasculated floret and 2 with non–emasculated florets), were used for treatment and the remaining four pots were used for control. The panicles of all the plants in all the three sets were sprayed with 12.33 per cent crude garlic extract with a pneumatic hand sprayer at 4 pm. Immediately after garlic extract spray, panicles of one of the sets were inoculated with freshly harvested ergot conidia to run–off point. The second and the third sets were similarly inoculated with ergot conidia 24 and 48 h after garlic spray. All the sets were maintained in a greenhouse at 25±2°C and 85–95 per cent rh. Number of infected and healthy florets in each treatment were counted 15 days after inoculation.

Effect of prophylactic spray in field. Ergot susceptible cultivar bulk Y was planted in 3 replications each with 2 rows of 4 m length at ICRISAT Patancheru during the 1993 rainy season. To minimise interplot interference during inoculation and garlic spray each of the 2–row treatment plot was surrounded by four rows of bulk Y. In each plot 20 panicles (10 panicles $line^{-1}$) of similar age were selected. At stigma emergence at tip, all the selected panicles were sprayed with crude garlic extract. In the first treatment, panicles were sprayed with 12 per cent garlic extract, 24 h after, the panicles were spray inoculated with ergot conidial suspension (1x10^6). In the second and the third treatment inoculations were done 48 and 72 h after garlic spray. Water sprayed controls were maintained for all the treatments. All the treated panicles were bagged with parchment paper bags. Fifteen days after inoculation, ergot severity (%) was recorded.

In an another experiment, garlic extract spraying and inoculation were done simultaneously. The experiment was conducted during the rainy and non–rainy

days of the 1993 rainy seasons, and also during the post–rainy seasons of 1992 and 1993. Other details were similar to the prophylactic spray in the field. Each treatment had three replications. Separate non–inoculated controls were maintained for each experiment.

Results and Discussion

Effect of garlic extract on conidial and pollen grain germination. There was 100 per cent inhibition of conidial germination in all the six concentrations of garlic extract compared to 100 per cent germination in control (Table 1). Germination of pollen grain ranged from 90 to 94 per cent in the three lower concentrations of the extract (9–14%).

Table 1. Germination (%) of conidia of ergot (*Claviceps sorghi*) and pollen grain of sorghum in different concentration of crude garlic extract at ICRISAT Patancheru

Garlic extract (%)		Germination (%)[a]	
		Conidia	Pollen grain
100	1:0	0	–[b]
50	1:1	0	–
33	1:2	0	–
20	1:3	0	–
16	1:5	0	94.2
12	1:7	0	90.3
9	1:9	0	91.7
Water control		100	97.6
SEm±		–	1.3867

[a]Mean of 3 repetitions each with 2 replications.
[b]Not tested.

Ergot control by prophylactic garlic spray in greenhouse. Garlic sprays protected both unemasculated and emasculated florets up to 48 h after spray (Table 2). Moderate to heavy ergot severity (27–66%) developed in the control pots. However, the ergot severity was more in emasculated treatment, particularly with simultaneous and 24 h after inoculations than in the unemasculated florets.

Ergot control by prophylactic garlic spray in field. Garlic spray remained effective for up to 72 h for controlling ergot in the field (Table 3). Unexpectedly, 48 h treatment was less effective than 72 h treatment.

Effect of garlic spray during the rainy and non–rainy days. Garlic sprays (12%) were highly effective in controlling ergot development during the post–rainy seasons. The reduction in ergot severity was by 86 per cent in 1992 and 69 per cent in 1993 post–rainy seasons. Interestingly, during the non–rainy days of 1993 rainy season the reduction in ergot severity was upto 75 per cent over control (Table 4). However, during the rainy days, the sprays were less effective.

Table 2. Effect of crude garlic extract (12%) spray on the development of sorghum ergot under greenhouse condition at ICRISAT Patancheru

Treatment	No. of florets		Ergot severity (%)	
	Observed	Infected	Treatment	Control
Simultaneous inoculation and garlic extract spray				
(a) Unemasculated	1171	0	0	34.8
(b) Emasculated	877	18	2	65.8
Inoculation 24 h after garlic spray				
(a) Unemasculated	1847	11	0.5	27.1
(b) Emasculated	780	0	0	38.0
Inoculation 48 h after garlic spray				
(a) Unemasculated	1198	0	0	47.8
(b) Emasculated	800	13	1.5	49.5
SEm±	216.124	3.0207	0.3061	4.4725

Table 3. Effect of crude garlic extract (12%) as prophylactic spray for the control of ergot (*Claviceps sorghi*) in the field conditions during 1993 rainy season at ICRISAT Patancheru

Treatment	Ergot severity[a] (%)	
	Treatment	Control
Spray 24 h before ergot inoculation	8.33 (5–20)[b]	78.80 (60–90)
Spray 48 h before ergot inoculation	16.60 (10–25)	90.00 (60–90)
Spray 72 h before ergot inoculation	3.75 (0–10)	79.00 (70–90)
SEm±	5.8132	14.0621

[a]Mean of three replications each with 20 panicles.
[b]Data inside the parentheses are ergot severity (%) range of 20 panicles.

The results show that garlic possesses fungicidal properties and it can be used to control ergot in sorghum. There was nearly 100 per cent control under greenhouse conditions (in the absence of external inoculum) but it was comparatively less effective under field conditions

where continual supply of inoculum was available in addition to inoculation. Also, the spray was less effective during the rainy–days than during the non–rainy days. It was due to the infection that had occurred after the rains had washed down the spray residues. Addition of an adhesive may reduce this problem to some extent.

Table 4. Effect of crude garlic extract (12%) spray for the control of sorghum ergot (*Claviceps sorghi*) during the rainy and rain–free days of 1993 rainy season and post–rainy seasons of 1992 and 1993 at ICRISAT Patancheru

	Ergot severity[a] (%)			
	Rainy days	Rain free days	Post–rainy season	
Treatment	1993	1993	1992	1993
Garlic spray at stigma emergence at tip followed by ergot conidial spray inoculation	57.18 (50–70)	9.11 (5–15)	4.6 (0–15)	6.7 (0–20)
Control (Only ergot conidial spray inoculation)	90.57 (80–100)	84.37 (70–95)	90.6 (76–98)	75.8 (50–100)
SEm±	34.2142	18.4983	20.7949	16.5557

[a]Mean of three replications each with 20 panicles.
Figures in parentheses are the ergot severity ranges.

Flowering event in sorghum, taking 7–11 days that makes the availability of pollen grain for longer period, is a major problem in obtaining a high level of ergot control. Therefore, the timing of spray will greatly effect the level of ergot control. Our limited experience shows that spraying should be done at emergence of stigma at the tip of panicles and that entire panicles should be covered with the spray. The residual effect of garlic remained effective for three days under rainfree conditions. However, the effect may stay for still longer period.

In our studies, the extract selectively inhibited 100 per cent conidial germination even in the lowest concentration of 6 per cent without adversely affecting pollen grain germination. However, the extract showed toxic effect on panicles in concentration of more than 10 per cent under field conditions. It is possible that different varieties of garlic, grown under different soil and agroclimatic conditions, may show different levels of fungicidal efficacy and/or toxicity on different cultivars. Therefore, there is a need for considerable research on these factors in order to develop an appropriate spray schedule.

In the past systemic fungicide benomyl has been used to control sorghum ergot. There is a need to compare the usefulness of garlic with fungicide keeping in view of their cost, availability and environmental pollution. With all the factors being equal, garlic should be more acceptable not because of its non–toxicity to the spray operator but also because it is environmental friendly. Which is in line with the current thinking of protection of environment from pollution. The garlic spray protects the crop from infection rather than curing the disease. Its advantages are its simplicity and safety. It does not interfere with pollination. The farmers can buy the raw material cheaply in the local market. The spray is nearly 100 per cent effective if used prior to infection in the post–rainy season. The most popular chemical fungicide mancozeb is at best 75 per cent effective.

Garlic, possesses more than 200 different compounds, including medicinally important properties. It has been used by people as preserver and restorer of health and youth (Dalvi and Solunkhe, 1993); as an excellent carminative, a nerve tonic and antiseptic in Hindu medicine (Aman, 1969); and in the treatment of tuberculosies, asthama, diarhoea, impotency, paralysis etc. (Dalvi and Solunkhe 1993). It has also been used as suppressor of cholesterol and triglycerides, reducer of blood clotting, and anticarcinogenic to laboratory animals. Despite knowing its antimicrobial, antifungal and anti insecticidal properties, which are ascribed to allicin – a break down compound of allicin, garlic has not been assessed widely for the control of plant diseases.

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