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Bioassay of Fungicides against Collar Rot Disease of Groundnut

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ABSTRACT: Groundnut is an important oilseed crop in India. Groundnut crop faces challenges because of the many diseases. Among them collar rot caused by *Aspergillus* spp. is one of the severe disease which causes great economic yield losses. Collar rot is a seed-borne disease which reduce the germination by rotting of seeds. Both systemic and non-systemic fungicides can be used as seed treatment for effective management of disease. So, bioefficacy of five fungicides *viz.*, mancozeb, carboxin + thiram, hexaconazole, tebuconazole and mancozeb + carbendazim were tested against *A. niger, A. flavus* and *A. terreus* at four varied concentrations by using poison food technique to find out the best fungicide and its concentration for managing the disease. Among all the fungicides tested, mancozeb + carbendazim proved to be more effective followed by carboxin + thiram, hexaconazole and mancozeb.

Keywords: Collar rot, fungicides, groundnut, poison food technique, seed-borne.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) belongs to leguminaceae family is an important food and oilseed crop originated from South America. It is cultivated in tropical and subtropical countries as a sole crop and intercrop extensively in both *Kharif* and *Rabi* seasons with an average rainfall requirement of 600 to 1200 mm. In the world, groundnut is cultivated in more than 90 countries (Virmani and Singh 1985). Nearly 30.19 M ha area in the world is dedicated to groundnut production whereas, in India it is 6.0 M ha with a production of 6.7 MMT (USDA, 2021). India holds first place in area and second place in the production of groundnut in the world. In Andhra Pradesh, it is cultivated in an area of 1.0 M ha with a production of 0.6 MT and 595 kg ha^{-1} productivity (Ministry of Agriculture and Farmers' Welfare, 2016-17). This crop encounters more than 70 diseases during its production. Among them collar rot poses greater threat to crop with an estimated yield loss to be up to 70% (McDonald *et al*., 1985; Lukose *et al*., 2008). Collar rot is widespread in almost all groundnut growing states of India *viz.*, Andhra Pradesh, Tamil Nadu, Gujarat, Punjab, Uttar Pradesh, Maharashtra, Karnataka, Rajasthan and Orissa. This disease appears at two stages *viz.*, pre-emergence and post-emergence stages. Because of the infection to seed in the soil, the emerging hypocotyls will be rotted by sooty black mass of spores in the pre-emergence stage. On cotyledons circular light brown lesions will be seen initially and advances to hypocotyl or stem as water soaked lesions in the post-emergence stage. Rotting of succulent hypocotyls will lead to collapsing and death of seedlings. Being a seed borne disease, it leads to abortion, shrinking, discolouration, size reduction, physiological alternation, rotting and reducing the germination percentage of seeds. It is more prevalent in soils with low moisture content and high temperature, approximately 30ºC (Kishore *et al*., 2007). The pre-emergence stage will incur upto 15 per cent whereas the post-emergence stage accounts for upto 2 per cent losses (Rasheed *et al*., 2004). Collar rot can be managed by seed treatment with both non-systemic and systemic chemical fungicides like companion, bavistin, vitavax power, steam, raxil and kavach (Rathod *et al.*, 2010; Rakholiya *et al.*, 2012; Nandeesha *et al.*, 2013; Srinivasan *et al.*, 2015; Kumari *et al.*, 2016; Rakesh *et al*., 2017). Carbendazim at 500 ppm and carboxin at 1000 ppm concentration completely inhibited the mycelial growth up to 100 per cent of test pathogen (Rohtas *et al*., 2016). Carbendazim + mancozebat 200 ppm inhibited 94.38% growth of the collar rot pathogen (Sekhon *et al*., 2019). Carbendazim + Mancozeb, Tebuconazole + Trifloxystrobin and Propiconazole

showed complete inhibition at all the concentrations (250, 500, 750 & 1000 ppm) in poison food technique against collar rot pathogen (Saran *et al*., 2022). The present study focuses on the evaluation of effect of different fungicides against *Aspergillus* spp. under *in vitro* conditions and selecting the best chemical for the management of collar rot disease.

MATERIALS AND METHODS

The bioassay of fungicides against test pathogen involved use of five different fungicides and virulent isolates of test pathogen *i.e., A. niger, A. flavus* and *A. terreus* following poisoned food technique *in vitro* (Nene and Thapliyal 1993). To perform the test, the amount of fungicides to be used is obtained using standard formulae and the same is mixed with PDA at lukewarm temperature. For each 9 cm Petriplates, 20 ml of poisoned PDA was added and allowed for solidification. Later, the solidified media in the Petriplates was inoculated with three day old test pathogen isolates as 5 mm culture discs aseptically in the center using cork borer and dissection needle. The control plates containing PDA without fungicides were inoculated with the test pathogen following the procedure mentioned as above. Three replicas of each fugicidal treatment and control were incubated for seven days at 28 ± 2˚C in the BOD incubator. Per cent growth inhibition was calculated in each treatment by comparison with control plates.

The per cent inhibition was measured by using the formula:

$$I = \frac{C - T}{C} \times 100$$

Where in,

I = Per cent inhibition of mycelial growth.

C = Colony diameter of pathogen in control (mm).

T = Colony diameter of pathogen in treatment (mm).

Table 1: List of fungicides and concentrations used for the study.

Sr.No.	Fungicides	Trade name	Recommended dose	Concentration (ppm)	Source of supply
1	Mancozeb 75% WP	Hyzeb M-45	0.25 %	700,1400,2000,2500.	Hyderabad Chemical Private Ltd
2	Carboxin 37.5% +Thiram 37.5% DS	Vitavax power	0.2 %	500,1000,1500,2000.	DhanukaAgritech Ltd
3	Hexaconazole 5% SC	Hexadhan plus	0.2 %	500,1000,1500,2000.	DhanukaAgritech Ltd
4	Tebuconazole 2 DS	Raxil	0.1 %	250,500,750,1000.	Bayer Crop Science Limited
5	Mancozeb 63% + Carbendazim 12% WP	Companion	0.25 %	700,1400,2000,2500	Indofil Industries Limited

Design: CRD Number of replications - 3

Observations recorded: The colony diameter in treatments will be measured when the control plates are filled with fungal growth.

RESULTS AND DISCUSSION

Antifungal activity of 5 chemicals was assayed *in-vitro* by poison food technique for *Aspergillus* spp. Results revealed that all the fungicides were capable of inhibiting growth of test fungus at recommended dosage as compared to control (Plates 1-5).

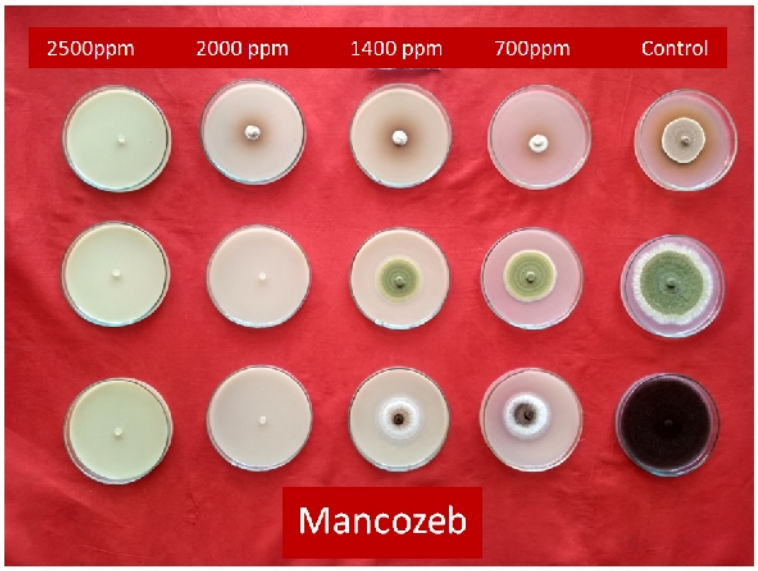

Plate 1. Evaluation of bioefficacy of mancozeb against *A. niger*, *A. flavus* and *A. terreus* by poison food technique.

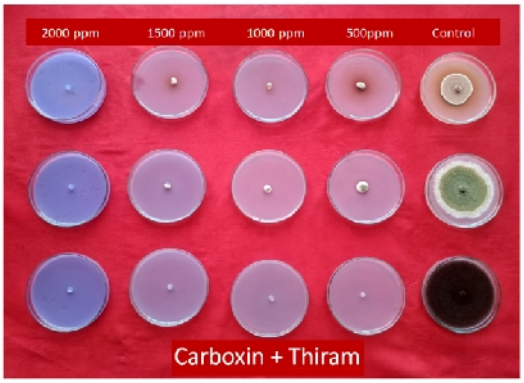

Plate 2. Evaluation of bioefficacy of Carboxin + Thiram against *A. niger*, *A. flavus* and *A. terreus* by poison food technique.

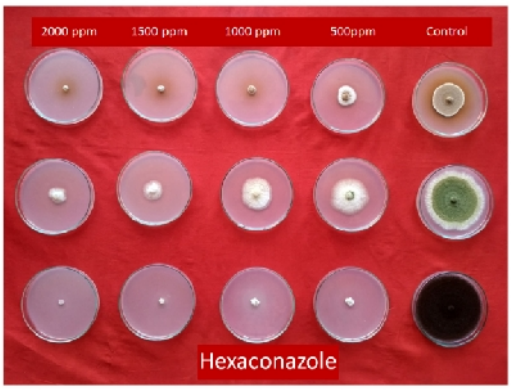

Plate 3. Evaluation of bioefficacy of Hexaconazole against *A. niger*, *A. flavus* and *A. terreus* by poison food technique.

Plate 4. Evaluation of bioefficacy of Tebuconazole against *A. niger*, *A. flavus* and *A. terreus* by poison food technique.

Plate 5. Evaluation of bioefficacy of Mancozeb + Carbendazim against *A. niger*, *A. flavus* and *A. terreus* by poison food technique.

Evaluation of effect of fungicides against *A. niger in vitro*. The data (Table 2 and Fig. 1) revealed that, among the different concentrations of mancozeb tested, maximum inhibition (100.00 %) was recorded at 2500 and 2000 ppm which were on par with each other and significantly differed with remaining concentrations and least was recorded at 700 ppm (25.60 %). However, carboxin + thiram exhibited complete inhibition at 2000, 1000, 1500 ppm and least was recorded at 500 ppm (90.56 %) which was significantly differed with all the concentrations. Whereas, hexaconazole showed cent per cent inhibition at 1500 and 2000 ppm which were significantly differed with remaining concentrations and lowest was recorded at 500 ppm (84.26 %) followed by at 1000 ppm (86.41 %). While tebuconazole at 1000 ppm recorded significantly highest inhibition (100.00 %) followed by 750 ppm (86.33 %) and 500 ppm (70.00 %). Lowest was recorded at 250 ppm (34.44 %). Whereas, mancozeb + carbendazim exhibited 100 per cent inhibition at all the concentrations tested *i.e.*, 700, 1400, 2000, 2500 ppm.

Table 2: Evaluation of bioefficacy of fungicides against *A. niger* using poisoned food technique.

Sr. No.	Treatments	Concentrations (ppm)	Radial growth (cm)	% Inhibition
1.	**Mancozeb75% WP**	700	6.70	25.56(30.36)^{i}
		1400	6.20	31.11(33.89)^{h}
		2000	0.00	100.00(90.00)^{a}
		2500	0.00	100.00(90.00)^{a}
2.	**Carboxin37.5% +Thiram 37.5% DS**	500	0.85	90.56(72.08)^{b}
		1000	0.00	100.00(90.00)^{a}
		1500	0.00	100.00(90.00)^{a}
		2000	0.00	100.00(90.00)^{a}
3.	**Hexaconazole 5% SC**	500	1.42	84.26(66.60)^{d}
		1000	1.22	86.41(68.34)^{c}
		1500	0.00	100.00(90.00)^{a}
		2000	0.00	100.00(90.00)^{a}
4.	**Tebuconazole 2 DS**	250	5.90	34.44(35.92)^{g}
		500	2.70	70.00(56.77)^{f}
		750	1.50	83.33(65.88)^{e}
		1000	0.00	100.00(90.00)^{a}
5.	**Mancozeb 63% + Carbendazim 12% WP**	700	0.00	100.00(90.00)^{a}
		1400	0.00	100.00(90.00)^{a}
		2000	0.00	100.00(90.00)^{a}
		2500	0.00	100.00(90.00)^{a}
6.	**Control**	-	9.00	0.00(0.00)^{j}
	C.D(P=0.05)		0.02	0.20(0.16)
	SEm(±)		0.01	0.07(0.06)
	SE(d)		0.01	0.10(0.08)
	C.V(%)		0.64	0.15(0.14)

*values in parenthesis are angular transformed values

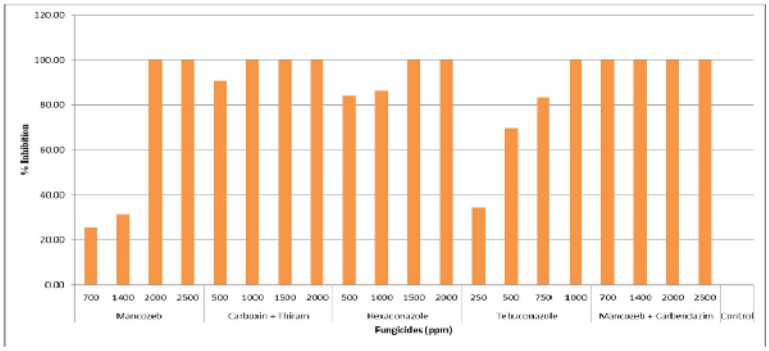

Fig. 1. Evaluation of bioefficacy of fungicides against *A. niger* using poisoned food technique.

Among the fungicides tested, mancozeb + carbendazim proved to be completely effective in inhibition of mycelial growth at all concentrations. Tebuconazole (at 1000 ppm), carboxin + thiram (at 1000 ppm), hexaconazole (at 1500 ppm), mancozeb (at 2000 ppm) showed cent per cent inhibition**.**

Evaluation of effect of fungicides against *A. flavus in vitro.* From the data in Table 3 and Fig. 2 it is revealed the among the concentrations tested, mancozeb showed complete inhibition at 2500 and 2000 ppm which were on par with each other and significantly differed with remaining concentrations. The mycelial growth inhibition decreased with decreasing the concentrations of fungicide (33.59 to 27.98 %) from 1400 to 700 ppm. In case of carboxin + thiram, significantly maximum mycelial growth inhibition (100.00 %) was recorded at 2000 ppm followed by at 1500 ppm (90.91 %) and 1000 ppm (84.47 %). Least inhibition (77.83 %) was recorded at 500 ppm. While hexaconazole exhibited significantly highest mycelial growth inhibition (66.13 %) at 2000 ppm. The inhibition was decreased with decrease in concentration (61.78 % to 33.59 %) from

1500 to 500 ppm. Whereas, tebuconazole exhibits significantly highest inhibition at 1000 ppm (75.10 %) followed by 750 ppm (42.69 %) and 500 ppm (39.13 %). Lowest was recorded at 250 ppm (27.27 %). However, in case of mancozeb + carbendazim complete inhibition (100.00 %) was recorded at all the concentrations tested *i.e.*, 700, 1400, 2000 and 2500 ppm.

Table 3: Evaluation of bioefficacy of fungicides against *A. flavus* using poisoned food technique.

Sr. No.	Treatments	Concentrations (ppm)	Radial growth (cm)	% Inhibition
1.	Mancozeb 75% WP	700	6.07	27.98(31.92)^{m}
		1400	5.60	33.59(35.41)^{k}
		2000	0.00	100.00(90.00)^{a}
		2500	0.00	100.00(90.00)^{a}
2.	Carboxin 37.5% + Thiram 37.5% DS	500	1.87	77.83(61.88)^{d}
		1000	1.31	84.47(66.76)^{c}
		1500	0.77	90.91(72.43)^{b}
		2000	0.00	100.00(90.00)^{a}
3.	Hexaconazole 5% SC	500	5.60	33.59(35.14)^{k}
		1000	5.07	39.92(39.17)^{i}
		1500	3.22	61.78(51.79)^{g}
		2000	2.86	66.13(54.39)^{f}
4.	Tebuconazole 2 DS	250	6.13	27.27(31.47)^{l}
		500	5.13	39.13(38.70)^{ij}
		750	4.83	42.69(40.78)^{h}
		1000	2.10	75.10(60.04)^{e}
5.	Mancozeb 63% + Carbendazim 12% WP	700	0.00	100.00(90.00)^{a}
		1400	0.00	100.00(90.00)^{a}
		2000	0.00	100.00(90.00)^{a}
		2500	0.00	100.00(90.00)^{a}
6.	Control	-	8.43	0.00(0.00)^{o}
	C.D(P=0.05)		0.07	1.04(0.65)
	SEm(±)		0.02	0.36(0.23)
	SE(d)		0.04	0.51(0.32)
	C.V(%)		1.50	0.94(0.66)

*values in parenthesis are angular transformed values

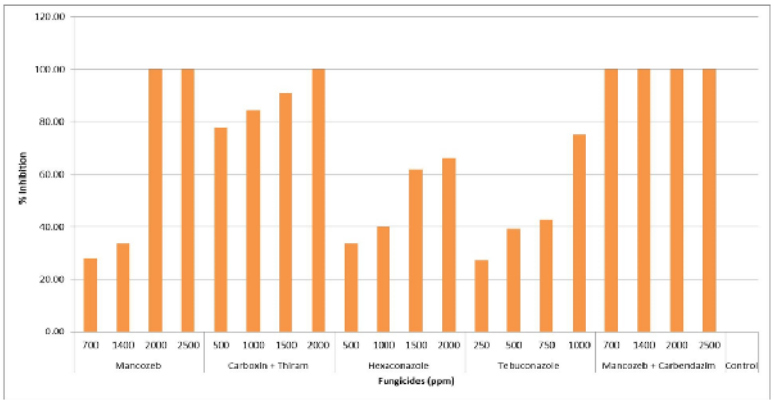

Fig. 2. Evaluation of bioefficacy of fungicides against *A. flavus* using poisoned food technique.

Among the fungicides tested, mancozeb + carbendazim was proved to be more effective in inhibiting the mycelial growth of the *A. flavus* followed by mancozeband carboxin + thiram.

Evaluation of effect of fungicides against *A. terreus in vitro.* It is evident from the Table 4 and Fig. 3 that, mancozeb at 2500 ppm showed 100 per cent inhibition of growth of pathogen followed by 2000 ppm (61.60 %) and 1400 ppm (56.03 %). The lowest was recorded at 700 ppm (47.43 %). In case of carboxin + thiram, cent per cent inhibition was recorded at 2000 ppm, whereas 1500 ppm and 1000 ppm recorded 78.87 per cent and 75 per cent respectively. But least per cent of inhibition was recorded at 500 ppm *i.e.*, 68.93. However, hexaconazole recorded significantly maximum per cent inhibition (100.00 %) was recorded at 2000 ppm followed by at 1500 ppm (78.53 %) and 1000 ppm (66.13 %). The least inhibition percentage was recorded at 500 ppm with 31.67 %. While tebuconazole showed highest growth inhibition (61.17 %) at 1000 ppm. The per cent inhibition was reduced with reduction in concentration (30.17 to 2.60 %) from 750 to 250 ppm. But, mancozeb + carbendazim inhibited cent per cent growth of *A. flavus* at all concentrations tested *i.e.*, 700, 1400, 2000, 2500 ppm.

Among all the tested fungicides, the results revealed that mancozeb + carbendazim found to be more effective against *A. terreus* followed by carboxin +

thiram, hexaconazole and mancozeb. In all the treatments, as the concentrations of fungicide increases there was a parallel decrease in the growth of test pathogen.

The results were also in accordance with Andge *et al.* (2017) who studied the efficacy of five fungicides *viz.*, carbendazim, thiram, carboxin + thiram, carbendazim + thiram and carbendazim + mancozeb for management of *A. niger* under *in vitro* conditions. They found that all fungicides showed 100 per cent inhibition of test fungus at all concentrations. Whereas, Rakesh *et al.* (2017) studied the bioefficacy of six fungicides *viz.*, cabendazim, mancozeb, thiram, tebuconazole, vitavax + thiram and cabendazim + mancozeb on the mycelial growth of *A. niger in vitro* and found that carbendazim + mancozeb, vitavax + thiram and tebuconazole showed 100 per cent inhibition at recommended dosage and vitavax + thiram showed cent per cent inhibition at half recommended dosage were also corroborated to our study. While Rani *et al.* (2017) tested the bioefficacy of six fungicides *viz.*, metalaxyl, thiram, mancozeb, mancozeb + carbendazimazoxystrobin and tebuconazole for control of *A. niger* under *in vitro* conditions and observed that tebuconazole showed maximum inhibition (100.00 %) at recommended and half recommended dosage and mancozeb showed minimum inhibition (43.24 %) at recommended dosage. Similar results were obtained by Vineela *et al.* (2018) who tested the *in vitro* efficacy of ten different chemicals against *A. niger* by inhibition zone technique and revealed that maximum antifungal activity was shown by thiophanate methyl (100.00 %) followed by tebuconazole (60.60 %), difenconazole (50.80 %), azoxystrobin (49.69 %). Saran *et al*. (2022) documented that Carbendazim + Mancozeb, Tebuconazole + Trifloxystrobin and Propiconazole showed 100 per cent mycelial growth inhibition at all the concentrations (250, 500, 750 & 1000 ppm) in poison food technique which was also corroborated to our research finding.

Table 4: Evaluation of bioefficacy of fungicides against *A. terreus* using poisoned food technique.

Sr. No.	Treatments	Concentrations (ppm)	Radial growth (cm)	% Inhibition
1.	**Mancozeb 75% WP**	700	2.03	47.43(43.51)^{j}
		1400	1.70	56.03(48.45)^{i}
		2000	1.50	61.60(51.69)^{g}
		2500	0.00	100.00(90.00)^{a}
2.	**Carboxin 37.5% + Thiram 37.5% DS**	500	1.20	68.93(56.10)^{e}
		1000	0.97	75.00(59.98)^{d}
		1500	0.83	78.87(62.61)^{b}
		2000	0.00	100.00(90.00)^{a}
3.	**Hexaconazole 5% SC**	500	2.63	31.67(34.22)^{k}
		1000	1.30	66.13(54.39)^{f}
		1500	0.83	78.53(62.38)^{bc}
		2000	0.00	100.00(90.00)^{a}
4.	**Tebuconazole 2 DS**	250	3.77	2.60(9.28)^{n}
		500	3.27	15.50(23.14)^{m}
		750	2.70	30.17(33.30)^{kl}
		1000	1.50	61.17(51.43)^{gh}
5.	**Mancozeb 63% + Carbendazim 12% WP**	700	0.00	100.00(90.00)^{a}
		1400	0.00	100.00(90.00)^{a}
		2000	0.00	100.00(90.00)^{a}
		2500	0.00	100.00(90.00)^{a}
6.	**Control**	-	3.87	0.00(0.00)^{o}
	C.D(P=0.05)		0.07	1.61(1.09)
	SEm(±)		0.02	0.56(0.38)
	SE(d)		0.03	0.79(0.54)
	C.V(%)		3.12	1.48(1.13)

*values in parenthesis are angular transformed values

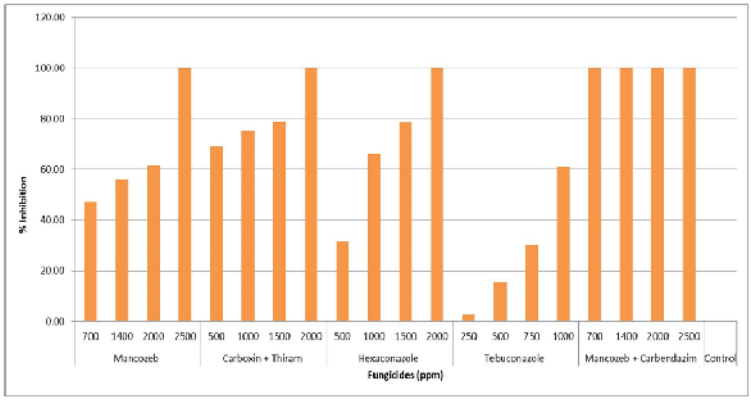

Fig. 3. Evaluation of bioefficacy of fungicides against *A. terreus* using poisoned food technique.

CONCLUSION AND FUTURE SCOPE

The use of chemicals has been believed to be a rapid and potential control measure against fungal diseases. Collar rot of groundnut caused by *Aspergillus* spp. is one of the severe disease that causes great economic losses. In the current study, the different fungicides have been tested against *Aspergillus* spp. Among all the fungicides tested against *A. niger*, *A. flavus* and *A. terreus* at four varied concentrations by using poison food technique, mancozeb + carbendazim proved to be more effective followed by carboxin + thiram, hexaconazole and mancozeb. Further, these effective fungicides can be used for seed treatment in combination with potential bioagents after compatibility studies as an integrated disease management practice.

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Conflict of Interest. None.

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