Evaluation of different fungicides against afla rot, *Aspergillus flavus* Link in groundnut

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Summary

Four systemic, four non-systemic and two combined fungicides at different concentrations were tested *in vitro* through poison food technique against *Aspergillus flavus* (Link), a causal organism of afla rot of groundnut. All the fungicides with their respective concentration were found inhibitory to the radial growth of *A. flavus*. Among all the systemic fungicides the highest growth inhibition of 99.99 per cent was recorded with tebuconazole 25% EC at all concentration (100, 250 and 500 ppm) followed by carbendazim 50% WP at 500 ppm (99.99%), 250 ppm (85.64%) and 100 ppm (82.64%) and hexaconazole 5% EC at 500 ppm (99.99%), 250 ppm (84.75%) and 100 ppm (77.58%). Among non-systemic fungicides, the highest growth inhibition of 90.88, 86.01 and 81.19 per cent were recorded with mancozeb 75% WP at 1500, 1000 and 500 ppm concentration, respectively. Among the combined fungicides, the highest growth inhibition 99.99 per cent was recorded with Carbendazim 12% + Mancozeb 63% WP at concentrations of 1500, 1000 and 500 ppm, respectively.

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Introduction

Groundnut (*Arachis hypogaea* L.), also known as peanut, earthnut, monkey nut, manilla nut, pinda, goober and kingpin of oilseeds is an annual legume crop. It is the thirteenth most important food crop of the world and third most important oil seed crop used for vegetable oil production (Upadhyaya and Dwivedi 2015). More than a hundred different countries across the tropics, subtropics, and warm temperate zones around the world cultivate groundnuts (Upadhyaya et al. 2012).

Globally, Groundnut covers 327 lakh hectares with a production of 539 lakh tonnes with a productivity of 1648 kg per hectare (FAO 2021). With annual all-season coverage of 54.2 lakh hectares, India ranks first in Groundnut area under cultivation and is the second largest producer in the world with 101 lakh tonnes with productivity of 1863 kg per hectare in 2021-22. In *kharif* 2022-23, groundnut production was 83.69 lakh tonnes (1st advance estimates) in an area of 45.53 lakh hectares (Department of Agriculture and Farmers Welfare, 2022). Groundnut is cultivated in one or more (*kharif*, *rabi* and summer) seasons, but nearly 90% of acreage and production comes from *kharif* crop (June-October). Gujarat is the largest producer contributing 36 per cent of the total production of Groundnut, followed by Rajasthan (17 %), Tamil Nadu (7.5 %) (Directorate of Economics and Statistics, 2022). Gujarat is the major groundnut-producing state in the country. The sowing area of Groundnut in the year 2021-22 is 1.99 million hectares and production is 4.49 million MT. Significant increase in sowing area during *kharif* season resulted in an increase in Groundnut production. In the year 2021-22, Groundnut production increased by 22.64 % with respect to the normal production of the state. In Gujarat, Devbhoomi Dwarka, Junagadh, Banaskantha, Rajkot, Amreli, Jamnagar, Bhavnagar and Sabarkantha are major groundnut-producing states (Directorate of Agriculture 2022). Groundnut seeds (kernels) contain 35.8- 54.2 per cent oil (Jambunathan et al. 1985; Maheshwari et al. 2022), 16.2-36.0 per cent protein (Dwivedi et al. 1990) and 10-20 per cent carbohydrate (Salunkhe et al. 1992).

Groundnut crop suffers from major diseases such as early leaf spot (*Cercospora arachidicola* Hori), late leaf spot (*Passalora personata* Berk. Curt. N. Arx), rust (*Puccinia arachidis* Speg), stem rot (*Sclerotium rolfsii* Sacc.), root rot [*Macrophomina phaseolina* (Tassi) Goid.], collar rot (*Aspergillus niger* Van Tieghem), afla rot (*Aspergillus flavus* Link), nematode disease like root-knot and viral diseases like stem necrosis, bud necrosis, mottle and clum (Ghewande & Reddy 1986; Konjengbam & Devi 2020).

Among the soil-borne diseases, afla rot caused by *Aspergillus flavus* is an important disease in the Gujarat region and also in groundnut-growing areas of the world (Klich 2007). *Aspergillus flavus* is a pathogenic fungus in the phylum Ascomycota. *A. flavus* is most common in warm temperate zones and environments with low water levels and higher temperatures. *A. flavus* is found globally as a saprophyte in soils and causes disease in many important agriculture crops including yellow mould in groundnut in the field, preharvest, postharvest, storage and during transit. There are 18 known analogues of aflatoxins with three series being significantly important from a food safety perspective B-series (AFB_1 and AFB_2), G-series (AFG_1 and AFG_2) and M-series (AFM_1 and AFM_2). *A. flavus* and *A. parasiticus* are the major producers of aflatoxins, whereby the *A. flavus* produce B-series aflatoxins. The “B” and “G” refer to the blue and

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green fluorescence color produced under UV light, while the subscript numbers indicate major and minor compounds, respectively (Jallow et al. 2021). AFB_1 is classified as a Group 1 carcinogen by the IARC (1993) due to the sufficient evidence of its involvement in cancer development in humans. Temperature, relative humidity, moisture content and drought stress during the harvesting are the main factors that determine the ability of *A. flavus* to grow during storage (Waliyar et al. 2015). Globally, FAO (The Food and Agricultural Organization of the United Nations) has provided regulations on mycotoxin content in both food and feeds, and FDA (Food and Drug Administration) has assigned specific limits for aflatoxins for human consumption, *i.e.*, 20 ppb (parts per billion) and 0.5 ppb in food and dairy products respectively. Aflatoxin contamination in agricultural crops may lead to an annual loss of more than US$ 750 million in Africa (Gbashi et al. 2018). In the USA, aflatoxin contamination leads to an annual income loss of more than US$ 100 million (Coulibaly et al. 2008).

Material and Methods

The experiment was conducted in factorial CRD (Completely Randomized Design) with factor A (Treatments) and factor B (Concentration) to study the efficacy of different fungicides (Table 1) on the growth of *A. flavus in vitro* by poison food technique. For this purpose, 250 ml PDA medium was poured into sterilized glass conical flasks of 500 ml capacity. The calculated quantity of tested fungicides was added aseptically in a conical flask, and then the flask containing fungicide were mixed well by stirring to facilitate uniform mixing of fungicides in the medium. Afterwards, 20 ml of medium with respective fungicide was poured into previously sterilized 9 cm (90 mm) diameter Petri plates labelled and allowed to solidify. Three repetitions were maintained in each treatment. The Petri plates containing PDA without the addition of fungicides serve as a control. The fungal block of 5 mm diameter was kept aseptically in the centre of each Petri plate from seven-day old culture of *A. flavus* grown on PDA with the help of sterilized cork borer and inoculated at 27±2°C temperature.

Table 1. List of fungicides were evaluated *in vitro* against *A. flavus*

S. No.	Common name	Concentration (ppm)		
Systemic fungicides				
T_1	Carbendazim 50% WP	100	250	500
T_2	Tebuconazole 25% EC	100	250	500
T_3	Difenoconazole 25% EC	100	250	500
T_4	Hexaconazole 5% EC	100	250	500
Non-systemic fungicides				
T_5	Mancozeb 75% WP	500	1000	1500
T_6	Copper oxychloride 50% WP	500	1000	1500
T_7	Chlorothalonil 75% WP	500	1000	1500
T_8	Propineb 70% WP	500	1000	1500
Combined fungicides				
T_9	Carbendazim 12% + Mancozeb 63% WP	500	1000	1500
T_{10}	Metalaxyl 8% + Mancozeb 64% WP	500	1000	1500
T_{11}	Control	-	-	-

Observations recorded

When the control plate was entirely filled with fungal growth (90 mm), observation of radial growth of the fungus in all treatment was recorded and the per cent growth inhibition (PGI) was calculated by using the formula given by Vincent (1947).

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

Where,

PGI= Per cent growth inhibition

C= Radial growth of the pathogen in control (mm)

T= Radial growth of the pathogen in treatment (mm)

Results and Discussion

The results presented in Tables 2, 3 and 4 indicated that all the fungicides evaluated significantly reduced the growth of *A. flavus* as compared to the control, but all the fungicides and their concentration significantly differ within themselves. Among all the concentrations, a higher concentration of each fungicide produced maximum inhibition of *A. flavus*.

Evaluation of fungicides against *A. flavus in vitro*

All four systemic fungicides were found inhibitory to the mycelial growth of *A. flavus*. Irrespective of concentrations (100, 250 and 500 ppm), exerted a significant effect with mean growth inhibition expressed by various systemic fungicides ranging from 81.24 to 99.99 per cent. The highest mean growth inhibition of 99.99 per cent was recorded with tebuconazole 25% EC. The next best fungicides in order of merit were carbendazim 50% WP, hexaconazole 5% EC and difenoconazole 25% EC with respective mean growth inhibition of 89.42, 87.44 and 81.24 per cent, respectively. The results presented in Table 3 revealed that all four non-systemic fungicides were found inhibitory to the mycelial growth of *A. flavus*. Among them, the highest mean growth inhibition of 86.03 % was recorded with mancozeb 75% WP followed by propineb 70% WP (75.66%), whereas least in chlorothalonil 75% WP (63.66%) followed by copper oxychloride 50% WP (64.86%). Two combined fungicides were found inhibitory to the mycelial growth of *A. flavus* (Table 3). The highest mean growth inhibition of 99.99 per cent was recorded with Carbendazim 12% + Mancozeb 63% WP, followed by Metalaxyl 8%+ Mancozeb 64% WP (76.84%).

Table 2. *In vitro* evaluation of systemic fungicides against *A. flavus*

S. No.	Fungicides	Growth inhibition (%) Concentration (ppm) 100	250	500	Mean
T_1	Carbendazim 50% WP	65.37^{c} (82.63)	67.74^{b} (85.64)	89.39^{a} (99.99)	**74.16^{b} (89.42)**
T_2	Tebuconazole 25% EC	89.39^{a} (99.99)	89.39^{a} (99.99)	89.39^{a} (99.99)	**89.39^{a} (99.99)**
T_3	Difenoconazole 25% EC	60.27^{d} (75.41)	65.72^{bc} (83.10)	67.38^{bc} (85.21)	**64.46^{c} (81.24)**
T_4	Hexaconazole 5% EC	61.74^{d} (77.58)	67.02^{bc} (84.75)	89.39^{a} (99.99)	**72.72^{b} (87.44)**
T_5	Control	4.05^{e} (0.50)	4.05^{e} (0.50)	4.05^{e} (0.50)	**4.05^{d} (0.50)**
Mean		56.16^{c} (67.22)	58.78^{b} (70.80)	67.92^{a} (77.14)	-
		Fungicide	**Concentration**		**Fungicide × Concentration**
S. Em. ±		0.40	0.31		**0.70**
C.D. at 5%		1.16	0.90		**2.01**
C.V.%		**1.98**			

Figures in parentheses are retransformed values of arc sine transformed values.
Treatment means with the letter(s) in common are not significant by DNMRT at 5% level of significance.

Table 3. *In vitro* evaluation of non-systemic fungicides against *A. flavus*

S. No.	Fungicides	Growth inhibition (%) Concentration (ppm) 500	1000	1500	Mean
T_1	Mancozeb 75% WP	64.30^{c} (81.19)	68.03^{b} (86.01)	72.43^{a} (90.88)	68.25^{a} (86.03)
T_2	Copper oxychloride 50% WP	50.95^{hi} (60.31)	53.08^{gh} (63.92)	57.00^{f} (70.34)	53.68^{c} (64.86)
T_3	Chlorothalonil 75% WP	49.56^{i} (57.92)	53.68^{gh} (64.92)	55.63^{fg} (68.14)	52.96^{c} (63.66)
T_4	Propineb 70% WP	58.30^{ef} (72.38)	60.95^{de} (76.42)	62.16^{cd} (78.19)	60.47^{b} (75.66)
T_5	Control	4.05^{j} (0.50)	4.05^{j} (0.50)	4.05^{j} (0.50)	4.05^{d} (0.50)
Mean		45.43^{c} (54.46)	47.96^{b} (58.36)	50.26^{a} (61.61)	-
		Fungicide	**Concentration**	**Fungicide × Concentration**	
S.Em. ±		0.52	0.40	0.90	
C.D. at 5%		1.50	1.16	2.59	
C.V.%		3.24			

Figures in parentheses are retransformed values of arc sine transformed values.
Treatment means with the letter(s) in common are not significant by DNMRT at 5% level of significance.

Table 4. *In vitro* evaluation of combined fungicides against *A. flavus*

S. No.	Fungicides	Growth inhibition (%) Concentration (ppm) 500	1000	1500	Mean
T_1	Carbendazim 12% + Mancozeb 63% WP	89.39^{a} (99.99)	89.39^{a} (99.99)	89.39^{a} (99.99)	**89.39^{a} (99.99)**
T_2	Metalaxyl 8% + Mancozeb 64% WP	55.76^{d} (68.34)	60.79^{c} (76.18)	68.04^{b} (86.01)	**61.53^{b} (76.84)**
T_3	Control	4.05^{e} (0.50)	4.05^{e} (0.50)	4.05^{e} (0.50)	**4.05^{c} (0.50)**
Mean		49.73^{c} (56.27)	51.41^{b} (58.89)	53.83^{a} (62.17)	-
		Fungicide	**Concentration**		**Fungicide ×**

			Concentration
S.Em. ±	0.14	0.14	**0.24**
C.D. at 5%	0.42	0.42	**0.72**
C.V.%	**0.82**		

Figures in parentheses are retransformed values of arc sine transformed values.
Treatment means with the letter(s) in common are not significant by DNMRT at 5% level of significance.

Effect of different fungicide concentrations on *A. flavus*

It was evident from the data that irrespective of the fungicides (*i.e.* systemic, non-systemic or combined), the inhibitory effect was increased positively with the increasing concentration. The systemic fungicides were evaluated at three different concentrations *viz*., 100, 250 and 500 ppm. Among them, the highest mean inhibition of 77.14% was recorded with 500 ppm concentration followed by in 250 ppm (70.80%) and 100 ppm (67.22%). The non-systemic fungicides were evaluated at three different concentrations *viz*., 500, 1000 and 1500 ppm. Among them the highest inhibition of 61.61% was recorded with 1500 ppm concentration, followed 1000 ppm (58.36%) and 500 ppm (54.46%). The combined fungicides were evaluated at three different concentrations *viz*., 500, 1000 and 1500 ppm. Among them, the highest inhibition of 62.17% was recorded with 1500 ppm concentration followed by 1000 ppm (58.89%) and 500 ppm (56.27%).

Interaction effect of fungicide and concentration on *A. flavus*

The interaction effect of fungicides and concentrations was also found significant. In case of systemic fungicides (Table 2) the highest growth inhibition of 99.99 per cent was recorded with tebuconazole 25% EC at all concentrations (100, 250 and 500 ppm), carbendazim 50% WP and hexaconazole 5% EC at 500 ppm concentration followed by carbendazim 50% WP (85.64%) at 250 ppm, whereas least growth inhibition was found in difenoconazole 25% EC (75.41%) at 100 ppm which was at par with hexaconazole 5% EC (77.58%) at 100 ppm. The data (Table 3) revealed that the highest growth inhibition (90.88%) was recorded in mancozeb 75% WP at 1500 ppm, followed by 1000 ppm (86.01%), whereas the least in chlorothalonil 75% WP (57.92%) at 500 ppm which was at par with copper oxychloride 50% WP (60.31%) at 500 ppm concentration. The data (Table 4) revealed that the highest growth inhibition 99.99 per cent was recorded with carbendazim 12% + mancozeb 63% WP at all concentrations (500, 1000 and 1500 ppm) followed by metalaxyl 8%+ mancozeb 64% WP (86.01%) at 1500 ppm.

From the presented data of systemic, non-systemic and combined fungicides tested against *A. flavus in vitro* suggested that in systemic fungicides, tebuconazole 25% EC at all concentrations, carbendazim 50 WP and hexaconazole 5 EC at 500 ppm concentration, in non-systemic fungicide mancozeb 75% WP at all concentration, propineb 70% WP at 1500 ppm concentration, whereas in combined fungicides carbendazim 12% + mancozeb 63% WP were most effective to inhibit the fungal growth of *A. flavus*. Sudha et al. (2013) studied an integrated approach for the management of aflatoxin contamination in chilli and they found that among the systemic fungicides, carbendazim showed the highest inhibition (73.00%) while among the contact fungicides maximum inhibition (91.1%) was observed with 0.3% mancozeb followed by captan (85.2%). Nathawat and Partap (2014) reported that the cent per cent growth inhibition was recorded in tabuconazole 2 DS and propiconazole 25 EC at 100 to 1000 ppm, respectively and the next effective fungicides were carbendazim 50 WP (500, 750 & 1000 ppm), difenconazole 25 EC (750 & 1000 ppm), a combination product of carbendazim 12% + mancozeb 63% 72 WP and captan 75 WP (1500, 2000 & 2500 ppm) which were also inhibited cent per cent growth of fungus. Kumar et al. (2005) recorded the highest growth inhibition (91.10%) with mancozeb at 0.3% concentration followed by captan (85.2%), among the systemic fungicides carbendazim (73%) at 0.15% concentration found most effective. Similar result was obtained by Rathod *et al*. (2010) and Nayak *et al*. (2018). Mancozeb is classified by the Fungicide Resistance Action Committee as a mode of action group M (multi-site) fungicide and it interferes with enzymes containing sulphydryl groups, disrupting several biochemical processes within the fungal cell cytoplasm and mitochondria. Carbendazim is a widely used, systemic, broad-spectrum benzimidazole fungicide and a metabolite of benomyl and these compounds interfere with mycelial growth and affect conidia formation and also inhibit spore germination of *A. flavus*. Azole compounds prevent the synthesis of ergosterol, a major component of fungal plasma membranes, by inhibiting the cytochrome P-450-dependent enzyme lanosterol demethylase (also referred to as 14-αsterol demethylase or P-450DM) and the result of that ergosterol depletion and accumulation of 14-α-methylated sterols, this interferes with the "bulk" functions of ergosterol in fungal membranes and disrupts both the structure of the membrane and several of its functions such as nutrient transport and chitin synthesis. The net effect is the inhibition of fungal growth (Shapiro et al. 2011).

Declaration of Interests

The authors have no conflict of interest to declare.

Data Sharing

All relevant data are within the manuscript.

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