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In Vitro Evaluation of Fungicides against *Aspergillus niger* causing Collar Rot Disease in Groundnut (*Arachis hypogaea* L.)

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A B S T R A C T

The present study was carried out to evaluate the efficacy of six fungicides against *Aspergillus niger* using two *in vitro* methods namely poisoned food technique and paper towel method. Poisoned food technique was carried out by making potato dextrose agar medium poisoned with different concentrations of fungicides followed by inoculation with actively growing culture of *A. niger*. Among the fungicides tested by using above method, tebuconazole at concentrations of 25, 50, 100 and 200 ppm and azoxystrobin at concentrations of 100 and 200 ppm, completely (100%) inhibited the mycelial growth of *A. niger* followed by carbendazim 25% + mancozeb 50% (Sprint) @ 200 ppm (94.38%). In paper towel method, two varieties of groundnut namely SG-99 and M-522 were tested. In this method, seeds from previously collar rot infected plants were taken and treated with fungicides, then wrapped between two paper towels and these were kept in incubator. The minimum seed per cent mortality and seed rots due to *A. niger* was observed in seed treatment with tebuconazole followed by azoxystrobin and carbendazim 25% + mancozeb 50% (Sprint) in both of the tested varieties. In both methods, three fungicides namely tebuconazole, azoxystrobin and carbendazim 25% + mancozeb 50% (Sprint) found most effective against *A. niger*.

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseeds and ancillary food crop in India with 7.4 million tonnes production from 4.7 million ha area and also has good export potential. India is the largest grower and second largest producer of groundnut after China with a national average productivity of about 1552 kg/ha (Anonymous, 2015). Major groundnut growing states in India are Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Rajasthan and Punjab. The productivity of groundnut in India is low in comparison to world average may be due to the damage caused by diseases and insect pests. Diseases cause considerable yield losses in groundnut. Fungal, virus and bacterial pathogens attack

the crop at various stages of growth and cause severe yield losses and in some cases impairing quality (Subrahmanyam *et al.,* 1980). The major soil borne diseases of groundnut caused by fungi are collar rot/crown rot/seedling blights (*Aspergillus niger*), stem rot/Sclerotium wilt (*Sclerotium rolfsii* Sacc.), alaroot (*Aspergillus flavus*) and dry root rot/dry wilt (*Macrophomina phaseolina*). Among all diseases, collar rot is reported to cause losses in yield up to 40% in India (Chohan, 1965). The losses may amount to 40-50% in terms of mortality of crop (Aulakh and Sandhu, 1970). In Gujarat, the incidence of seedling blight of groundnut was found as high as 50 per cent (Joshi, 1969).

Among various methods, fungicides serve as an important and efficient method to control collar rot disease caused by *Aspergillus niger*. From time to time, many workers made attempts to test the efficacy of different fungicides under *invitro* conditions. Wani and Kuruchave (2004) reported 100% inhibition of mycelium growth of *A. niger* and *A. flavus* by thiram and (carbendazim + mancozeb) at 250 ppm concentration.

Sharma *et al.,* (2012) observed that the carbendazim and mancozeb completely inhibited the mycelial growth of fungus at higher concentrations of 500 and 1000 ppm. Parjapati *et al.,* (2016) studied the efficacy of different fungicides against *A. niger* under *invitro* conditions.

They found that carbendazim (12%) + mancozeb (63%), azoxystrobin (18.2%) + difenconazole (11.4%), trifloxystrobin (25%) + tebuconazole (50%) and mancozeb (50%) + carbendazim (25%) at 500 and 100 ppm concentrations completely inhibited the mycelial growth of *A. niger*. Keeping these aspects in view, the present study was planned to evaluate the efficacy of different fungicides against *A. niger* under *in vitro* conditions.

Materials and Methods

The effect of six different fungicides namely mancozeb, tebuconazole, azoxystrobin, carbendazim, carbendazim 25% + mancozeb 50% (Sprint) and carboxin 37.5% + thiram 37.5% (Vitavax power) on the growth of *A. niger* was studied using Poisoned Food Technique and Paper Towel Method.

Poisoned food technique

Poisoned food assay (Nene and Thapliyal 1993) was employed to study efficacy of above mentioned fungitoxicants under lab conditions. Non-systemic fungitoxicant mancozeb was tested at a series of concentrations *viz*. 25, 50, 100, 200, 500 ppm, whereas systemic fungitoxicants (tebuconazole, azoxystrobin, carbendazim, carbendazim 25% + mancozeb 50% and carboxin 37.5% + Thiram 37.5%) were tested at series of concentrations *viz.* 25, 50, 100 and 200 ppm respectively. Required quantity of the test chemical was mixed with 100 ml of PDA sterilized medium and the poisoned medium was poured into Petri dishes (90 mm diameter) under aseptic conditions. Circular bits (7 mm) of the actively growing culture of fungus were placed aseptically in the center of each Petri dish and each concentration was replicated four times. The Petri dishes with PDA medium without fungicide served as control. After inoculation Petri Plates were incubated at 25±1°C. The radial colony growth of pathogen was recorded when the growth in untreated control plate was full (i.e. 90mm) and per cent inhibition in colony growth (Pi) was calculated by using formula devised by Vincent (1947).

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

Where,

C = Radial growth in control (mm)

T = Radial growth diameter in treatment

Paper towel method

The infected seeds of varieties SG-99 and M-522 were treated with six fungicides by soaking in solution of fungicides for 8 hours to evaluate the effect of different seed treatments on germination and seedling vigour by paper towel method. The fungicide solutions were made with concentrations of 0.30, 0.15, 0.10, 0.20, 0.40 and 0.30 per cent for mancozeb, tebuconazole, azoxystrobin, carbendazim, carbendazim + mancozeb and carboxin + thiram, respectively. The infected seeds were procured during *Kharif* 2016 from two groundnut varieties SG-99 and M-522 showing typical collar rot symptoms. Two types of controls were kept viz., infected seed with no seed treatment and apparently healthy seed with no seed treatment. Two sheets of paper towel were wetted by distilled water, leaving a 3cm margin.

Twenty five seeds were kept on it equidistantly in five rows each containing five seeds and sheet was covered with another moistened paper towel. Then paper towel was rolled, wrapped in wax paper and tied by rubber band. They were incubated at 28 ± 2 °C. Occasionally the sheets were kept moist.

Three replications were kept under each treatment. After 14 days sheets were opened to count number of normal seedlings, mortality (%) and seed rots (%). The categorization of normal seedlings, mortality (%) and seed rots (%) was done as described by Khare and Bhale (2000).

Data were recorded in terms of per cent germination, seedling length, seedling dry weight, vigour index length and vigour index mass using the following formulas:

$$\text{Germination (\%)} = \frac{\text{No. of normal seedlings}}{\text{Total seeds sown}} \times 100$$

Seedling length = Average length (cm) of 10 normal seedlings on the day of final count (after 14 days)

Seedling dry weight = Average weight (g) of 10 seedlings excluding the cotyledons after oven drying at 100^0 C for 24 hours

Vigour index length = Germination (%) × Seedling length (cm)

Vigour mass = Germination (%) × Seedling dry weight (g)

Results and Discussion

Evaluation of six fungicides were tested in laboratory by two methods namely poisoned food technique and paper towel method in Oilseed Plant Pathology Laboratory, Department of Plant Breeding and Genetics.

Poisoned food technique

Data with respect to inhibition of mycelia growth of *A. niger* of six different fungicides were recorded and results are presented in Table 1 and Fig 1. Data from the table revealed that, the efficacy of different fungicides, concentrations and their effect on the percent inhibition of mycelial growth of *A. niger* differed significantly. Among the test non-systemic fungicides, mancozeb was the most superior against *A. niger* as it provided the 56.38 per cent growth inhibition at 500 µg/ml. Singh and Singh (2006) reported the 100 per cent inhibition of the *A. flavus* at higher concentration (1000 µg/ml) of mancozeb. Similar results were also obtained by Sharma *et al.,* (2012). Among the test systemic fungicides, tebuconazole proved to

be the most effective by providing maximum mean per cent inhibition (93.04%) of colony growth followed by azoxystrobin (85.00%) (Plate 1). Tebuconazole at the concentration of 25, 50, 100 and 200 µg/ ml completely (100%) inhibited the colony growth of *A. niger*. However, azoxystrobin gave complete inhibition at concentrations of 100 and 200 µg/ ml. Results are in accordance with those reported by Raju and Naik (2006). They studied the 100% inhibition in the radial growth of *A. niger* with the fungicide tebuconazole at recommended and half the recommended concentrations. Nathawat and Partap (2014) also recorded complete inhibition of mycelial growth of *A. niger* by tebuconazole at concentration of 100 to 1000 µg/ ml. Among the two test combiproduct fungicides, carbendazim 25% + mancozeb 50% (Sprint) was proved to more effective by providing maximum mean per cent inhibition (58.93%). Sheth and Patil (2010) noted complete inhibition of mycelia growth of *A. niger* infecting citrus fruits by treating with carbendazim + mancozeb at 500 and 1000 µg/ml concentration. Kumari *et al.,* (2016) also obtained similar results. While, carboxin 37.5 + thiram 37.5 (vitavax power) gave mean colony growth inhibition of 49.67 per cent. Kumari *et al.,* (2016) found 65.80 per cent growth inhibiton of *A. niger* with carboxin 37.5 + thiram 37.5 (vitavax power).

In our present studies, 100 per cent inhibition in the radial growth of *A. niger* culture was obtained with two fungicides namely tebuconazole and azoxystrobin at 100 µg/ml concentration while carbendazim 25% + mancozeb 50% (Sprint) also gave good inhibition (94.38%) of *A. niger* at 200 µg/ml concentration.

Paper towel method

Effect of six fungicides as seed treatment was evaluated for germination, seedling length, dry weight, vigour index length and vigour index mass on groundnut varieties SG-99 and M-522. The results are given in Table 2. Significant variation with respect to per cent germination was recorded in different seed treatments. Among various chemicals, the maximum germination was observed in case of carbendazim 25% + mancozeb 50% (Sprint) in both the varieties SG-99 (96.33%) and M-522 (88.33%) followed by carboxin 37.5% + thiram 37.5% (Vitavax power). Seed treatments with carbendazim 25% + mancozeb 50% (Sprint) and carboxin 37.5% + thiram 37.5% (Vitavax power) were significantly superior over control (infected seeds) in respect of their effectiveness in increasing seed treatment. However, these two chemical seed treatments did not differ significantly from each other. Similar results were reported by Johnson and Subramanyam (2010). They observed that Vitavax power increased the seed germination significantly over other treatments and control. Likewise, Singh *et al.,* (2004) proved that the thiram increased seed germination and vigour of the groundnut seedlings.

The effect of various test seed treatments on seedling length, dry weight, vigour index length and vigour index mass is presented in Table 2. The seedling length (root length + shoot length) was increased significantly by carbendazim 25% + mancozeb 50% (Sprint) and carboxin 37.5% + thiram 37.5% (Vitavax power) over all other treatments with non-significant difference among them for both the varieties. In treatment done with Sprint, the seedling length was 24.13 cm (SG-99) and 20.12 cm (M-522) and in Vitavax power seedling length was 23.80 cm (SG-99) and 19.51 cm (M-522). While seedling length of seed treatment with mancozeb was at par with the infected control. Shivpuri *et al.,* (2011) observed that Vitavax power improved the shoot length of groundnut seedlings over the other treatments and control.

Table.1 Evaluation of different fungicides against *A. niger* under *in vitro* conditions by poisoned food technique

Systemicity	Fungicide	Per cent growth inhibition over check at different concentrations (µg ml^{-1})								
Non-systemic		**5**	**10**	**25**	**50**	**100**	**200**	**400**	**500**	**Mean**
	Copper Oxychloride	0.00 (0.00)	0.00 (0.00)	5.73 (13.84)	9.27 (17.72)	13.45 (21.51)	21.22 (27.42)	32.07 (34.48)	40.70 (39.62)	15.30
	Mancozeb	9.23 (17.68)	12.60 (20.78)	18.86 (25.73)	23.27 (28.83)	28.33 (32.15)	33.27 (35.21)	48.16 (43.93)	56.38 (48.65)	28.76
	Mean	4.61	6.30	12.29	16.27	20.89	27.24	40.11	48.54	
Systemic	Tebuconazole	73.88 (59.24)	84.34 (66.66)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	-	-	93.04
	Azoxystrobin	57.77 (49.45)	69.56 (56.49)	87.14 (68.96)	95.54 (77.78)	100 (89.96)	100 (89.96)	-	-	85.00
	Carbendazim	11.25 (19.59)	20.47 (26.89)	31.05 (33.85)	43.61 (41.31)	67.83 (55.42)	82.27 (65..07)	-	-	42.74
	Mean	47.63	58.12	72.73	79.72	89.27	94.09	-	-	
Combination (Systemic and Non-systemic)	Sprint (mancozeb 50 + carbendazim 25)	19.86 (26.45)	29.73 (33.03)	52.78 (46.57)	74.52 (59.66)	82.31 (65.10)	94.38 (76.26)	-	-	58.93
	Vitavax power (carboxin 37.5 + thiram 37.5)	14.31 (22.22)	24.37 (29.57)	38.88 (38.56)	61.12 (51.40)	72.77 (58.52)	86.24 (68.20)	-	-	49.61
	Mean	17.08	27.05	45.83	67.82	77.54	90.31	-	-	

Non systemic:
CD (p=0.05)
Fungicide = 0.40
Concentrations = 0.79
Fungicide x Concentrations= 1.13

Systemic:
CD (p=0.05)
Fungicide = 0.57
Concentrations = 0.80
Fungicide x Concentrations= 1.39

Combination (Systemic and Non systemic):
CD (p=0.05)
Fungicide = 0.67
Concentrations = 1.16
Fungicide x Concentrations= 1.64

Figures in parentheses are angular transformed value

Table.2 Effect of seed treatment with fungicides on germination and vigour of groundnut seed evaluated by paper towel method

Seed Treatment (Fungicide)	Dose (%)	Per cent Germination		Seedling length (cm)		Fresh weight of Seedlings (g/seedling)		Dry weight of Seedlings (g/seedling)		Vigour index length		Vigour index mass	
		SG 99	M 522	SG 99	M 522	SG 99	M 522	SG 99	M 522	SG 99	M 522	SG 99	M 522
Mancozeb	0.3	85.66 (67.76)	82.00 (64.89)	18.83	16.89	3.97	4.40	0.57	0.54	1509.56	1239.00	45.60	39.64
Tebuconazole	0.035	80.33 (63.95)	73.33 (59.05)	16.48	17.35	3.59	4.11	0.46	0.56	1414.54	1421.41	39.43	46.15
Azoxystrobin	0.1	86.33 (68.52)	80.33 (63.72)	19.02	17.95	3.95	4.19	0.52	0.59	1644.62	450.03	45.14	47.61
Carbendazim	0.2	94.66 (76.80)	85.33 (67.62)	23.48	18.25	4.38	4.48	0.59	0.60	2259.56	1560.81	56.82	51.21
Sprint	0.4	96.33 (81.01)	88.33 (70.17)	24.13	20.12	3.91	3.70	0.45	0.52	2300.35	1774.77	42.53	45.90
Vitavax power	0.3	95.33 (79.76)	84.33 (66.76)	23.80	19.51	3.99	4.11	0.52	0.55	2253.06	1647.37	49.55	46.40
Control (infected seeds)	--	74.66 (59.77)	70.66 (57.19)	15.77	13.18	3.55	3.64	0.46	0.47	1117.97	933.90	34.13	33.44
Control (Apparently healthy seeds)	--	85.00 (67.24)	76.00 (60.71)	17.81	15.91	3.67	3.81	0.48	0.50	1514.77	1209.18	40.77	37.97
CD at 5 % (Fungicide)	--	5.11		1.52		0.11		0.03		168.39		3.48	
CD at 5 % (Variety)	--	2.55		0.76		0.05		0.01		84.19		1.74	
CD at 5 % (Fungicide × Variety)	--	9.07		2.14		0.16		0.04		238.13		4.92	
CV (5%)	--	6.47		6.91		4.23		4.39		9.04		6.74	

Figures in parentheses are angular transformed values

Table.3 Germination status of groundnut seeds treated with different fungicides using paper towel method

Seed treatment	Dose (%)	Normal seedlings (%)		Mortality (%)		Seed rots (%)	
		SG 99	M 522	SG 99	M 522	SG 99	M 522
Mancozeb	0.3	48.67 (44.22)	47.67 (43.65)	37.33 (37.65)	38.67 (38.43)	14.67 (22.51)	17.33 (24.59)
Tebuconazole	0.035	88.00 (69.70)	74.67 (59.76)	8.00 (16.42)	10.67 (19.05)	4.00 (11.53)	12.00 (20.26)
Azoxystrobin	0.1	76.00 (60.64)	73.33 (58.89)	14.00 (21.96)	14.67 (22.51)	5.33 (13.35)	14.67 (22.51)
Carbendazim	0.2	54.67 (47.66)	52.00 (46.13)	22.67 (28.42)	28.00 (31.94)	18.67 (25.59)	26.67 (31.08)
Sprint	0.4	73.33 (58.89)	65.33 (53.91)	16.00 (23.57)	18.67 (25.59)	5.33 (13.35)	16.00 (23.57)
Vitavax power	0.3	70.67 (57.18)	52.00 (46.13)	21.33 (27.50)	21.33 (27.50)	13.33 (21.41)	20.00 (26.55)
Control (infected seeds)	--	22.67 (28.42)	17.33 (24.59)	52.00 (46.13)	53.33 (46.89)	25.33 (30.21)	29.33 (32.78)
Control (Apparently healthy seeds)	--	46.67 (43.07)	30.67 (33.61)	38.67 (38.43)	45.33 (42.31)	14.67 (22.51)	24.00 (29.32)
CD at 5 % (Fungicide)	--	3.15		2.84		2.91	
CD at 5 % (Variety)	--	1.57		1.42		1.46	
CD at 5 % (Fungicide × Variety)	--	4.46		4.01		4.12	
CV (5%)	--	5.53		7.82		10.72	

Figures in parentheses are angular transformed values

Fig.1 *Invitro* evaluation of different fungicides against *Aspergillus niger* by poisoned food technique

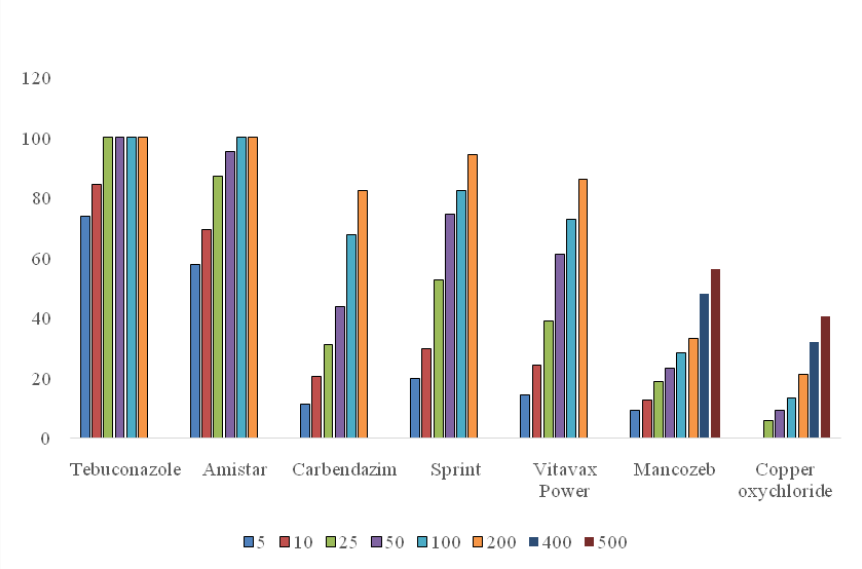

Fig.2 Germination status (%) of groundnut seeds (variety SG-99) treated with different fungicides using paper towel method

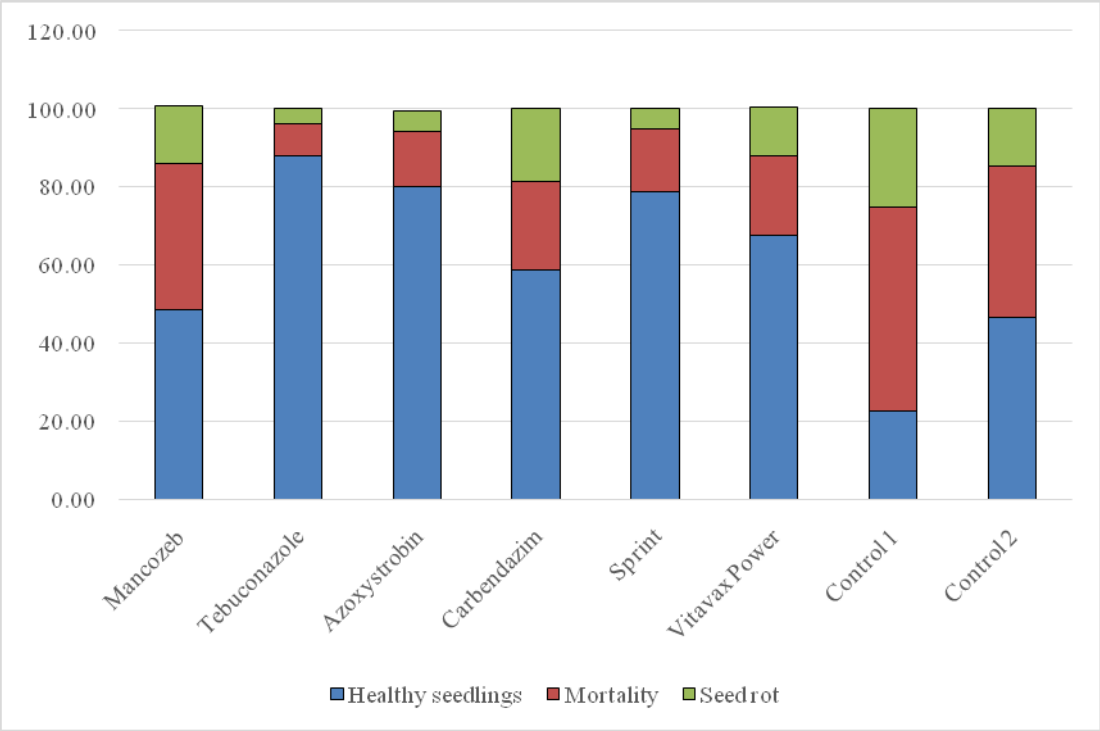

Fig.3 Germination status (%) of groundnut seeds (variety M-522) treated with different fungicides using paper towel method

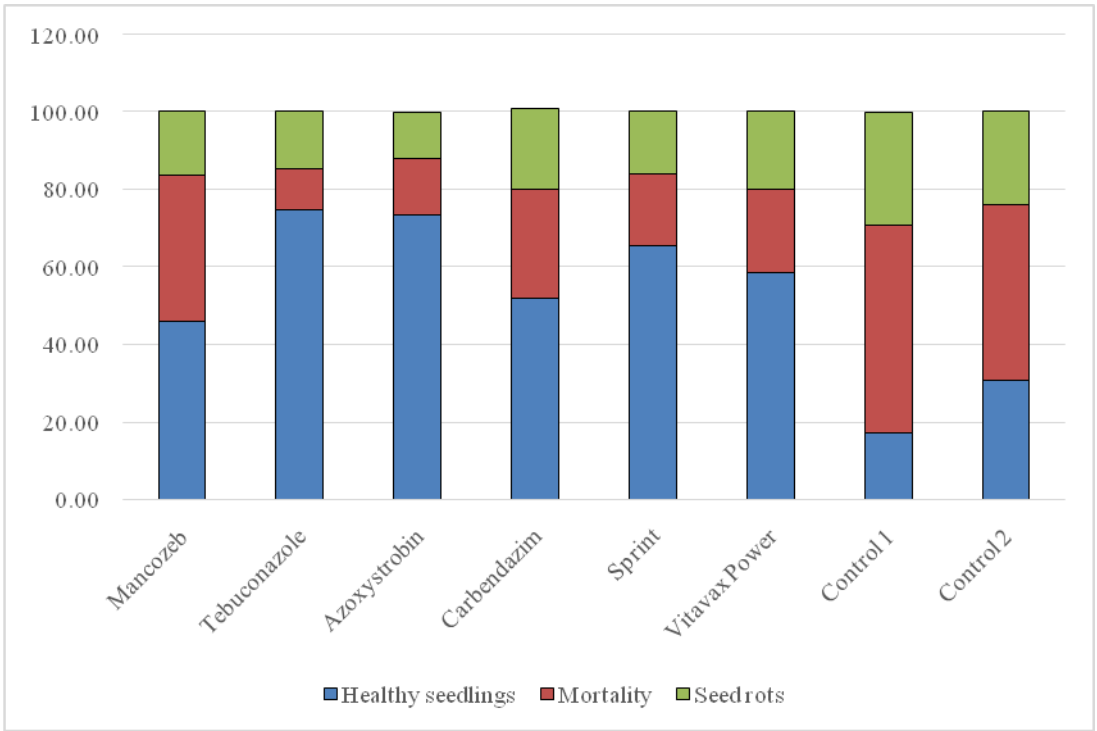

Plate.1 Colony growth inhibition of *Aspergillus niger* by (a) Tebuconazole and (b) Azoxystrobin

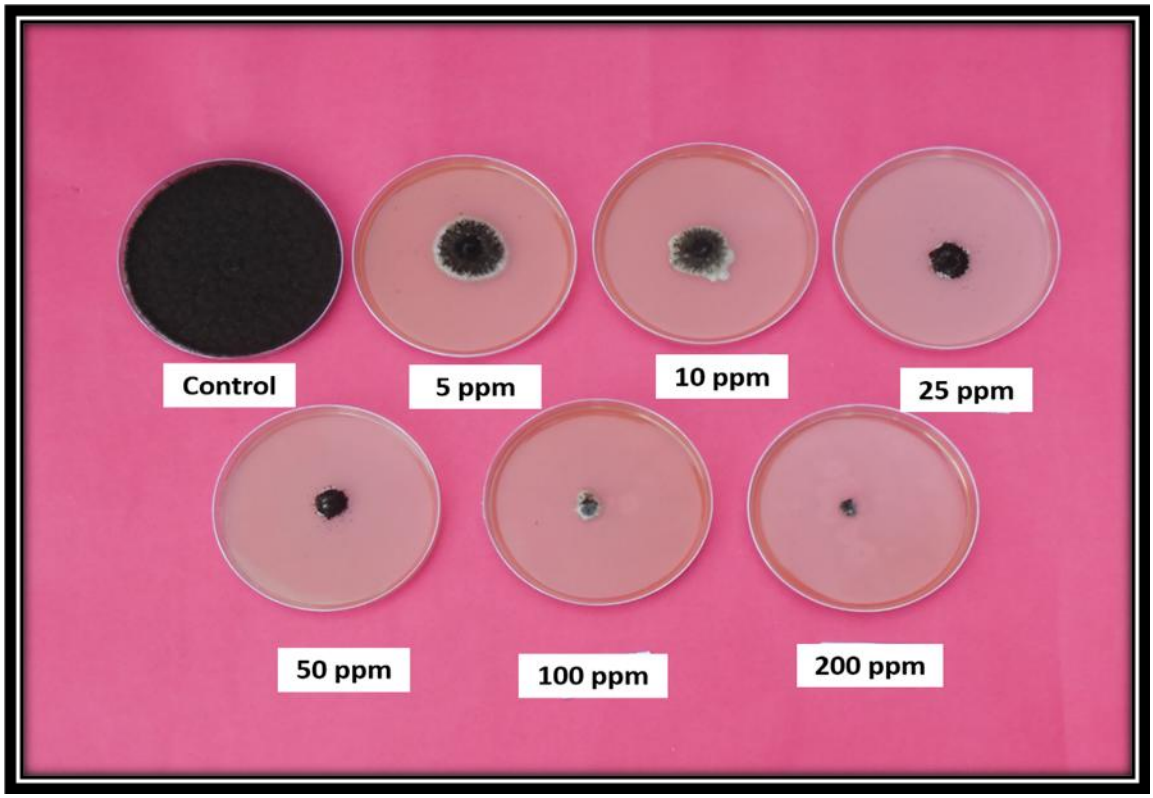

Plate.2 Vigour of groundnut seedlings (a) seed treatment with tebuconazole (b) untreated control using paper towel method

The vigour index in terms of length (cm/seedling) and dry weight (g/seedling) of seedlings was calculated to find effect of seed treatment on seedling vigour. The vigour index length was significantly higher in case of Sprint (2300.35 in SG-99 and 1774.77 in M-522) and Vitavax power (2253.06 in SG-99 and 1774.77 in M-522) over all other treatments with non-significant difference among them for both the varieties. The vigour index mass was significantly higher in case of carbendazim (56.82 for SG-99 and 51.21 for M-522) and Vitavax power over all other treatments with non-significant difference among them for both the varieties. Devi and Parsad (2009) observed that seed treatment with captan improved the growth parameters like yield and biomass production that was due to the increase in vigour of the seedlings. Shivpuri *et al.,* (2011) observed that Vitavax power improved the shoot length which ultimately gave the maximum vigour index length of groundnut seedlings over the other treatments and control.

The germination status of the chemically treated groundnut seeds (Fig 2 and 3) after 14 days of incubation in paper towel sheets is summarized in Table 3. The maximum percentage of healthy seedlings in variety SG-99 (88.00%) and M-522 (74.67%) was recorded in case of tebuconazole (Plate 2). The observations for the mortality (%) showed reverse trend, where minimum per cent mortality was recorded in tebuconazole in variety SG-99 (8.00 %) and M-522 (10.67 %) followed by azoxystrobin and Sprint. The minimum percentage of seed rots were found in seed treatments with tebuconazole (4.00% in SG-99 and 12.00% in M-522) and azoxystrobin (5.33% in SG-99 and 14.67% in M-522) for both the varieties. Johnson and Subramanyam (2010) evaluated the different fungicides and found that seed treatment with tebuconazole showed minimum collar rot occurrence. The observations summarized in Table 2 and 3 and Figures 2 and 3 led to the conclusion that the collar rot diseases has a marked effect on seed germination and seedling vigour. Seed treatment with

tebuconazole was the best followed by azoxystrobin and carbendazim 25% + mancozeb 50% (Sprint) to increase seed germination and seedling vigour both.

The above results from both the experiments revealed that tebuconazole was the best followed by azoxystrobin and carbendazim 25% + mancozeb 50% (Sprint) to control the collar rot of groundnut caused by *A. niger*.

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