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Evaluation of fungicides and herbicides against groundnut collar rot pathogen *Aspergillus niger* under *in vitro* conditions

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ABSTRACT

Collar rot of groundnut is caused by *Aspergillus niger* Van Tieghem. It is a soilborne pathogen, usually the occurrence of collar rot disease during the early stages of crop growth and results in high seedling mortality. The collar rot pathogen produces both seed rot (pre-emergence) and seedling blight symptoms (post–emergence). In case of seed rot, black masses of mycelium were seen on the surface of seed. Later, the pathogen causes rotting of seed. Rotten seed do not emerge from the soil. Collar rot affected seedlings show a circular brownish spot on cotyledon region. Later, the discoloured area rapidly disintegrates spreading to the stem and hypocotyl. The affected plants show general wilting, rotting just below the ground level. An experiment was conducted under *in vitro* conditions to evaluate the fungicides against the collar rot pathogen apart from these fungicides, some herbicides also evaluated to test their non-target effect against collar rot pathogen. A total of six fungicides and eight herbicides were evaluated by using poisoned food technique. Maximum inhibition (100%) of radial growth of *A. niger* was obtained with tebuconazole at recommended and half recommended dosage and least inhibition was obtained with mancozeb and azoxystrobin. Among herbicides pendimethalin was shown maximum inhibition (100%) against *A. niger* and least inhibition was observed with herbicide imazythapyr and imazythapyr + imazamox.

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is important oilseed crop. In India, Gujarat, Andhra Pradesh, Telangana, Karnataka and Tamil Nadu are the highest producers of groundnut. However, the production of

groundnut is decreasing day by day because of various biotic and abiotic stresses. Among these biotic factors like fungal diseases play a major role in the yield reduction (Ganesan and Sekar, 2012 and Mayee and Datar, 1988). The diseases caused by soilborne fungi are very important and several of them are potential yield reducers in certain regions. Among these soilborne diseases the collar rot caused by *Aspergillus niger* causing major havoc in groundnut growing areas and causing yield losses upto 26 per cent (Narain and Kar, 1990). Collar rot disease on groundnut seedlings was first reported by Jochem (1926) and in India it was first reported by Jain and Nema (1952) as *Aspergillus* blight. Collar rot disease is usually seen during the early stages of crop growth, and often results in seedling mortality at higher rates. The symptomatology of collar rot disease is commonly manifested as a pre-and post-emergence damping-off of the affected seedlings. In case of pre-emergence damping-off, seed rot is the most obvious symptom wherein, seeds are infected by the black spore mass of *A. niger*, thereby resulting in rotting, poor seed germination and crop stand. Post-emergence damping-off or seedling blight occurs on germinated seedlings and manifests as circular brownish spots on the cotyledons, followed by rotting and spreading of the disease to the collar and hypocotyl regions, resulting in sudden wilting and death of the plants. Occasionally, collar rot can continue upto crop harvesting stage resulting in damage to the seed (Gajera *et al.*, 2011). The fungus also produces heat canker symptoms (Boyle, 1953). Though, the annual yield losses due to collar rot alone are approximately 5 per cent, the disease has a potential to damage the crop with 40 per cent losses (Bakhetia, 1983). In India, collar rot is an economically significant disease and is reported from the states of Andhra Pradesh (Kulashreshta *et al.*, 1964), Punjab (Chohan, 1965 and Gupta and Chohan, 1970) and Rajasthan (Sharma *et al.*, 1973). In India, the losses may account to 40 to 50 per cent in terms of seedling mortality due to *A. niger* (Ghewande *et al*., 2002). Losses due to pre-emergence phase of the disease are upto 15 per cent, whereas post-emergence phase accounts to 2 per cent losses (Rasheed *et al*., 2004). Collar rot disease is more problematic in sandy soils (Gibson, 1953 and Chohan, 1965). Presently collar rot disease is managing through application of fungicides (Ezzahiri and Khattabi, 2004). Collar rot disease is usually managed through seed treatment with both systemic and non-systemic fungicides like tebuconazole, vitavax, thiram and mancozeb (Rakholiya *et al*., 2012). Apart from these fungicides some herbicides also showing non-target effect against the soilborne pathogens (Glaze *et al.*, 1984). Hence, the present study was under taken to test the *in vitro* efficacy of some fungicides and herbicides against collar rot pathogen.

MATERIAL AND METHODS

Poisoned food technique (Nene and Thapaliyal, 1993) was adopted to determine the inhibitory effect of fungicides and herbicides against *A. niger*. The required quantity of fungicides/ herbicides were calculated using standard formulas and were added to the PDA medium at lukewarm stage and thoroughly mixed before pouring into petriplates so as to get the desired concentration of active ingredient of each fungicide/ herbicide separately. The fungicides and herbicides were tested at recommended and half the recommended concentrations. Twenty ml of amended medium was poured in each of 90 mm sterilized Petri plates and allowed to solidify. Five mm discs of 3-day- old culture of test pathogen (*A. niger*) were cut with sterile cork borer and transferred aseptically to the centre of the poisoned medium. Simultaneously, control plates were maintained by placing 5 mm disc of test fungi in the centre of the non-poisoned PDA medium. Three replications were maintained in respect of each fungus and for each concentration. All the inoculated Petri plates were incubated at 28$\pm$ 2^{o}C in BOD incubator for seven days. The colony diameter in treatments were measured when the control plates was filled with fungal growth. Per cent growth inhibition was calculated in each treatment by comparison with control plates.

The per cent inhibition was measured by using the formula:

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

wherein,

I = Per cent inhibition of mycelial growth

C = Colony diameter in control (mm)

T = Colony diameter treatment (mm).

RESULTS AND DISCUSSION

A total of six fungicides were screened under *in vitro* conditions against *A. niger*. At recommended concentration, maximum inhibition (100%) of radial

growth was obtained with tebuconazole and least inhibition (43.24%) was obtained with mancozeb. This was followed by fungicide mancozeb+carbendazim (92.94%), thiram (83.92%), metalaxyl (63.13%) and azoxystrobin (48.62%). At recommended dosage, all these fungicides showed significant differences except azoxystrobin and mancozeb. For majority of the fungicides except tebuconazole (100% inhibition) a decrease in the pathogen inhibition was reported with a concomitant decrease in concentration (Table 1). At half recommended concentrations, fungicides showed inhibitory effects of fungicides were thiram (77.25%), mancozeb+carbendazim (67.01%), metalaxyl (57.64%), mancozeb (40.39%) and azoxystrobin (39.94%). At half recommended dosage except mancozeb and azoxystrobin remaining four fungicides were significantly different. Present results indicate that tebuconazole was highly effective in inhibiting *A. niger* at recommended and half the recommended concentrations (Plate 1 and 2).

Among all the herbicides (eight) evaluated, highest

Details of the fungicides used :
(1) Metalaxyl, (2) Mancozeb, (3) Azoxystrobin, (4) Tebuconazole, (5) Thiram, (6) Mancozeb + carbendazim

Plate 1 : Effect of fungicides on the radial growth of *Aspergillus niger* at recommended concentration under *in vitro* conditions

Details of the fungicides used :
(1) Tebuconazole, (2) Metalaxyl, (3) Azoxystrobin, (4) Thiram, (5) Mancozeb, (6) Mancozeb + carbendazim

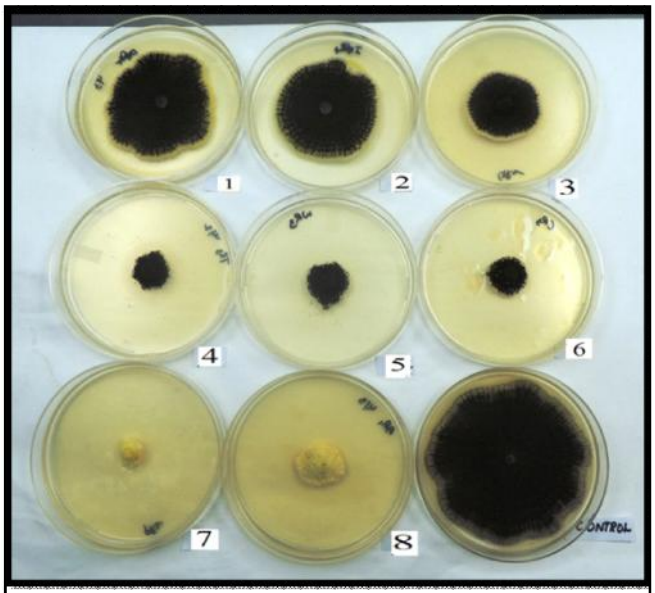
Plate 2: Effect of fungicides on the radial growth of *A. niger* at half recommended concentration under *in vitro* conditions

Table 1 : Evaluation of fungicides on the mycelial growth of *Aspergillus niger* causing groundnut collar rot under *in vitro* conditions

Fungicide	Per cent growth inhibition of *A. niger* over control		
	Concentration of fungicide		
	Recommended**		Half recommended
Azoxystrobin	48.62 (44.19)		39.94 (39.15)
Mancozeb	43.24 (41.09)		40.39 (39.44)
Metalaxyl	63.13 (52.63)		57.64 (49.38)
Mancozeb+Carbendazim	92.94 (80.85)		67.01 (54.93)
Tebuconazole	100.00 (90.00)		100.00 (90.00)
Thiram	83.92 (66.34)		77.25 (61.49)
Factors	C.D. (P=0.05)	SE(d)	S.E.±
Fungicides (A)	5.23	2.55	1.80
Concentrations (B)	2.79	1.36	0.96
(A x B)	7.40	3.61	2.55

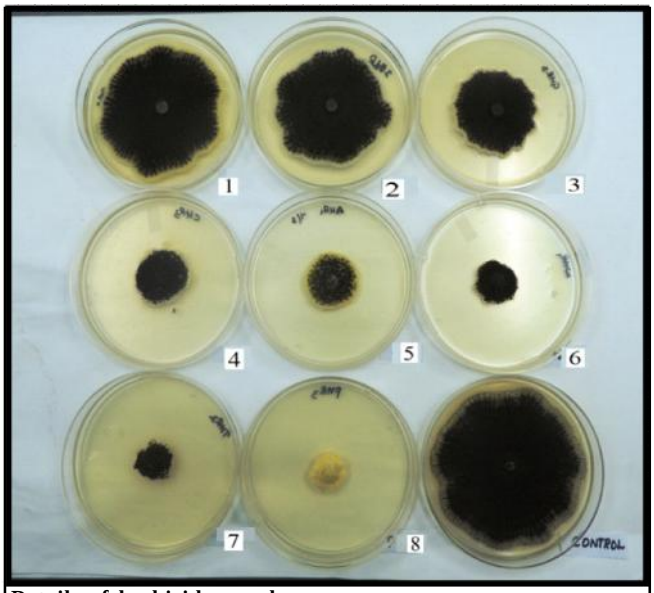
*Values in the parentheses are the angular transformed values and are means of three replications
**The recommended doses are 1000 ppm (azoxystrobin and tebuconazole);2000 ppm (mancozeb, metalaxyl, thiram) and 2500 ppm (mancozeb+carbendazim)

per cent inhibition (85%) of *A. niger* was obtained with pendimethalin at recommended dose. This is followed by quizalofop-p-ethyl (79.17%), cycloxydim (77.01%) and the difference between these two herbicides were non significant, followed by, fenaxoprop, propaquizafop and oxyflourfen with 75.42 per cent, 73.40 per cent and 52.43 per cent inhibitions, respectively. Significant difference was not found among these three herbicides. Least inhibition was observed with the herbicides imazethapyr and imazethapyr+imazamox were 28.19 per



Details of herbicides used :
(1) Imazethapyr+Imazamox, (2) Imazethapyr, (3) Oxyflourfen,
(4) Quizalofop-p-ethyl, (5) Fenaxoprop, (6) Cycloxydi
(7) Pendimethalin, (8) Propaquizafop

Plate 3 : Effect of different herbicides (at recommended dosage) on radial growth of *Aspergillus niger* under *in vitro* conditions



Details of herbicides used :
(1) Imazethapyr+Imazamox, (2) Imazethapyr, (3) Oxyflourfen,
(4) Cycloxydim, (5) Propaquizafop, (6) Fenaxoprop,
(7) Quizalofop-p ethyl, (8) Pendimethalin

Plate 4 : Effect of different herbicides (at half-recommended dosage) on radial growth of *Aspergillus niger* under *in vitro* conditions

Table 2 : Evaluation of herbicides on the mycelial growth of *Aspergillus niger* causing groundnut collar rot under *in vitro* conditions

Herbicide	Per cent inhibition of *A. niger* over control*		
	Concentration of herbicide		
	Recommended**		Half the recommended
Propaquizafop	73.40 (58.93)		66.18 (54.41)
Cycloxydim	77.01 (61.32)		61.66 (51.73)
Oxyfluorfen	52.43 (46.37)		43.47 (41.22)
Imazythapyr	28.19 (32.05)		25.62 (30.34)
Imazythapyr + Imazamox	21.92 (27.89)		14.03 (21.87)
Pendimethalin	85.41 (67.53)		75.16 (60.14)
Quizalofop- P- Ethyl	79.17 (62.82)		77.58 (61.71)
Fenaxoprop	75.42 (60.25)		70.62 (57.15)
Factors	C.D.(P=0.05)	SE (d)	S.E.±
Herbicides (A)	2.61	1.29	0.91
Concentrations (B)	2.84	0.60	0.43
(A x B)	3.70	1.82	1.29

*Values in the parentheses are the angular transformed values and are means of three replications
**The recommended doses are 430 ppm (Imazythapyr + Imazamox); 1500 (propaquizapop), 1700 ppm (oxyfluorfen), 2000 ppm (Imazythpyr, Fenaxoprop,Quizalopop- P- Ethyl); 3000 ppm (cyclodixim) and 5000 ppm (pendimethalin)

cent and 21.92 per cent, respectively and the differences between these herbicides were significant (Table 2). At half recommended dosage the inhibitory effects of quizalofop-p-ethyl and pedimethalin, were 77.58 per cent and 75.16 per cent and the treatments were on par. At half the recommended dosage the inhibitory effects of herbicides fenaxoprop, propaquizafop, Cycloxydim, oxyflourfen, imazethpyr and imazethpyr+imazamox ranged from 14.03 per cent to 70.62 per cent and all these herbicides differed significantly differ with each other (Plate 3 and 4).

In our present studies 100 per cent inhibition in the radial growth of *A. niger* was obtained with the fungicide tebuconazole at recommended and half the recommended concentrations. The results are in agreement with Raju and Naik (2006) and Nathawat and Pratap (2014) who reported 100 per cent inhibition in the radial growth of *A. niger* in onion and groundnut with triazole compounds like propiconazole, tebuconazole and difenconazole and also the combination product like mancozeb+carbendazim. In the present studies also 93 per cent inhibition was obtained with the combination product mancozeb+ carbendazim. The fungicides mancozeb and azoxystrobin were least effective in inhibiting the radial growth of *A. niger* but contradictory reports are also available with mancozeb where 100 per cent inhibition was obtained with this fungicide in groundnut against collar rot pathogen *A. niger* (Nandeesha *et al*., 2013).

Studies on effect of herbicides on pathogens are important because these herbicides not only inhibit the weeds but also show indirect effect on the growth of soilborne plant pathogens (Katan and Eshel, 1973; Altman and Campbell, 1977 and Papavizas and Lewis, 1979). In our studies the herbicide Pendimethalin was highly effective in inhibiting the radial growth of pathogen *A. niger* at its recommended and half-recommended dosage levels. Pendimethalin was also effective in inhibiting the radial growth of other soilborne fungi like *R. solani*. Complete inhibition of radial growth against these soilborne pathogens was observed under *in vitro* conditions by Bhoraniya *et al*. (2002) and Jhonson *et al*. (2008). In our studies herbicide quizalofop-p-ethyl also showed good level of inhibition (79.17%) next to pendimethalin in the growth of pathogen at its recommended dosage whereas, at half the recommended dosage the highest inhibition was found with quizalofop-p-ethyl followed by pendimethalin. Oxyflourfen also inhibited the radial growth of *A. niger* by 52.43 per cent and showed marked reduction in sclerotial production of *S. rolfsii* under *in vitro* conditions. Similar results was reported by Hua *et al*. (2002) in case of *R. solani* where the herbicide oxyflourfen reduced the sclerotial production. In the present studies, the herbicide imazethapyr and imazethpyr +imazamox were least effective in inhibiting the radial growth of *A. niger* which may due to the degradation of these herbicides by *A. niger*. Microbial degradation of herbicide glyphosate by *Aspergillus* sp. was studied by earlier workers (Eman *et al*., 2013). The herbicide pendimethalin was highly effective in inhibiting the radial growth of *A. niger* under *in vitro* conditions

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