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Management of Collar rot of Groundnut with Bio-agent, Botanicals and Chemicals

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Efficacy of pesticides was tested *in vitro* for the per cent mycelial growth inhibition of *A. niger*. Propiconazole, carbendazim and carboxin completely inhibited the mycelial growth up to 100 per cent at 200, 500 and 1000 ppm concentration, respectively as comparable with 86.94, 88.05 and 59.96 per cent at their respective 100, 200 and 500 ppm. Captan and thiram were found very less effective as they inhibited 81.11 and 72.77 per cent of fungal growth, respectively at higher concentration of 1000 ppm. Fungicide hexaconazole, herbicide pendimethalin and insecticide chlorpyriphos failed to show antifungal activity against *A. niger* even at 1000 ppm concentration. Three bio-agents *viz., Trichoderma viride*, *T. harzianum* and *Pseudomonas fluroscence* were tested for their inhibition of mycelial growth of *A. niger in vitro*. *T. viride* inhibited the mycelial growth up to 78.32 per cent followed by *T. harzianum* (72.50%), while the bacterial agent *P. fluroscence* only managed to inhibit 23.80 per cent of mycelial growth. Seed treatments with fungicides and soil inoculation with bio-agents significantly reduced the disease incidence. Among the botanicals, neem cake powder @ 20g/kg soil significantly controlled the disease up to 32.53 per cent.

Key words: Fungicides, mycelia growth, collar rot, *Aspergillus niger*.

Groundnut or peanut (*Arachis hypogaea* L.), is a very important legume crop of tropical and sub tropical areas of the world, described in 1753 by Linnaeus (Pattee and Young, 1982). In India, groundnut occupies 35 per cent of the total cropped area under oilseeds and accounts for 40 per cent of total oilseeds production (Anonymous, 2014). On an average, groundnut seed contains 45 per cent of oil and 26 per cent of protein and its kernels are relished either as snack, roasted or salted or raw form or also in the form of peanut butter. Obviously, poor soil fertility, abiotic and biotic stress factors limit the growth of groundnut crop and yield in many ways. Among biotic stresses, groundnut is attacked by many fungal, bacterial and viral pathogens. Collar rot caused by *Aspergillus niger* van Teighem is one of the most important disease of groundnut which is more extensive in the *kharif* than the *rabi*/*summer* seasons and causes more damage in sandy loam and medium black soil. Annual world yield loss caused by collar rot is more than 10 per cent (Pande and Rao, 2000) and is more prevalent in soils with low moisture content and high temperature, approximately 30ºC (Kishore *et al*., 2007). Several attempts have been made to control collar rot disease in groundnut by chemical means as seed dressers and foliar sprays application by various workers from time to time. Fungicides used prior to the mid 1950s were not effective against *A. niger*. In recent years, several fungicides have been reported to control the disease with varying degree of success (Desai and Bagwan, 2005). Shivpuri *et al*. (2011) evaluated four seed dressers against collar rot disease (*Aspergillus niger* van Teighem) of groundnut under field condition during *Kharif* 2008 to 2010. All seed treatments were found

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significantly superior over check in reducing pre and post emergence rot, increasing root and shoot length and pod yield. Vitavax Power @ 3.0 g/kg seed showed minimum incidence of pre and post emergence mortality i.e. 11.80% and 4.50%, respectively. Devi and Prasad (2009) used captan seed treatment as 2g/kg and reported the lowest disease incidence (64.7%) and higher germination (80.7%) under pot culture experiment compared to control exhibiting disease incidence and germination per cent as 97.7 and 75.3 per cent, respectively. Limited work has been reported in literature on management of this disease through the use of bio-agents and botanicals. Devi and Prasad (2009) reported that antagonistic nature of *Trichoderma* spp. in *vitro* against *A. niger.* The per cent inhibition of the pathogen was 68.0 per cent by *T. viride* followed by *T. harzianum* (54.0%) and *Pseudomonas fluorescens* (34.0%) and also seed treatment with *T. viride* @ 4g/kg in pot culture experiment, showed higher germination, disease incidence, shoot length, root length and dry matter production of 88.5 per cent, 72.3 35.3cm, 26.3 and 1.8g, respectively. Gajera *et al.* (2011) evaluated the twelve isolates of three Trichoderma strains in *vitro* against the collar rot. It was observed that *T. viride* 60 inhibited maximum (86.2%) growth of test fungus. Karthikeyan (1996) ascertained that soil application of the antagonist *T. viride* @ 500 g inoculum/20 sq.m, showed minimum disease incidence of 2.32 per cent as compared to control disease incidence of 10.40 per cent. Organic amendments are known to favour native antagonists and suppress soil and seed borne disease (Mayakrishnan, 1990; Alice, 1994). Since the pathogen is very difficult to manage due to its soil or seed borne nature and wide host range, therefore the present study was carried out with the objective to evaluation of different fungicides, bio-agent and botanicals for the control of Collar rot of groundnut caused by *Aspergillus niger* under *in vitro* and screen house conditions.

MATERIALS AND METHODS

Efficacy of different Fungicides and bio-agents against *A. niger in vitro*

Evaluation of fungicides/Pesticides

The Efficacy of six fungicides *viz.*, thiram, captan, carbendazim, carboxin, hexaconazole, propiconazole, chlorpyriphos (Insecticide) and pendimethalin (herbicide) on the growth of *Aspergillus niger* were tested under *in vitro* conditions using the standard procedure of poison food technique as given by Mayer (1962). Stock solution of each pesticide was prepared in double strength *i.e.* 50, 100, 200, 500 and 1000 ppm by dissolving weighed or measured quantity of pesticide in a measured volume of sterilized water. The double strength potato dextrose agar (PDA) medium was also prepared and sterilized at 15 lb pressure for 20 minutes. An equal volume of chemical solution and PDA was mixed in a sterilized conical flask and poured aseptically in the Petri plates. After solidification of medium, each Petri plate was centrally inoculated with 5 mm disc of fungus taken from 7 days old culture of *A. niger* with the help of sterilized cork borer and incubated at 28±1°C. Suitable controls were maintained for each chemical. Four replications of each pesticide were maintained and CRD was followed. Colony diameter of the fungus of each treatment along with control was measured (mm) and recorded after every 24 hours, till the test fungus occupied the full Petri plate in the controlled treatment. The per cent inhibition of mycelial growth over control was calculated by following formula given by Vincent (1947).

$$\text{Growth inhibition (\%)} = \frac{(C-T)}{C} \times 100$$

Where,

I = Per cent inhibition of mycelium growth

C = Radial growth of *A. niger* mycelium in control

T = Radial growth of *A. niger* mycelium in treatment

Evaluation of bio-agent

The antagonistic effect of three bio-agents *viz.*, *Trichoderma harzianum*, *Trichoderma viride* and *Pseudomonas fluorescens* on the growth of *A. niger* was tested using dual culture technique (Morton and Strouble, 1955). The fungus was cultured on the PDA while the bacteria were cultured on NDA but the antagonistic effects of the bio-agents were tested on PDA. Fifteen ml of liquefied PDA medium was poured into sterile Petri plates and allowed to solidify. From seven days old culture of *A. niger*, 5 mm mycelial disc was cut from the margin of the actively growing colony with a sterile cork borer and placed near the periphery, on one side of the PDA plate while an

antagonistic fungi were placed on the other side of the PDA plate just opposite to the first disc *i.e*. at an angle of 180º. Similarly, antagonistic bacteria obtained from three days old culture were streaked five cm long on the PDA medium at the two cm mark from the periphery of the Petri-dish. Simultaneously, five mm mycelial disc of *A. niger* were cut from the margin of the actively growing colony with a sterile cork borer and placed near the periphery on opposite side of the bacterial streak *i.e.* at an angle of 90º. All the plates were incubated at 28 ± 1 ºC for five days. Each treatment replicated four times as CRD and appropriate controls were maintained. The extent of antagonistic activity by fungal and bacterial antagonists was recorded on fifth day by measuring the growth of *A. niger* in dual culture plate and control plate. The per cent inhibition of *A. niger* was calculated as suggested by Vincent (1947) as described earlier for pesticides evaluation *in vitro.* The best treatments of both the fungicides and bio-agent evaluated *in vitro* were also tested for their efficacy under screen house conditions.

Efficacy of promising seed treating fungicides and bio-agents under screen house conditions

The antagonistic effect of the promising fungicides and bio-agents evaluated *in vitro* were further evaluated under the screen house conditions. These fungicides were: propiconazole (1ml/kg seeds), carbendazim (2g/ kg seeds) and captan (3g/ kg seeds) While the bio-agents were: *Trichoderma viride* (20g/kg soil) and *Trichoderma harzianum* (20g/kg soil). Soils were pre-inoculated with inoculum of *A. niger* in the earthen pots of 12" diameter. For the fungicides, seeds of each genotypes *viz.*, MH-4, MH-21, M-522, and HNG-10 were treated 72 hours prior to planting. For the bio-agents *Trichoderma viride*, and *T. harzianum*, they were initially grown on sterilized wheat medium by inoculating with 7 days old mycelium and incubated for 10 days at 28 ± 1 ºC to allow maximum establishment of the fungus mycelium. Prior to planting of seeds of each genotypes *viz*., MH-4, MH-21, M-522, and HNG-10, soils were inoculated with the respective bio-agents (20g/ kg soil).Ten seeds were planted in each pot and for each treatment four replications were maintained as CRD. Untreated seeds sown in pre-inoculated soil only with *A. niger* served as control. The observation was recorded 30 days after sowing

Table 1. Effect of different pesticides on per cent mycelial growth inhibition of *A. niger in vitro*

Pesticides	*Per cent mycelial growth inhibition at different concentrations (ppm)					
	50*	100*	200*	500*	1000*	Mean
Thiram	26.11 (30.71)	44.31 (41.72)	54.26 (47.42)	62.55 (52.24)	72.77 (58.52)	52.00 (46.12)
Captan	0.50 (4.05)	64.44 (53.37)	68.44 (55.79)	78.33 (62.23)	81.11 (64.21)	58.56 (47.93)
Carbendazim	68.33 (55.73)	83.65 (66.11)	88.05 (69.74)	100 (89.39)	100 (89.39)	88.00 (74.07)
Carboxin	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)	59.96 (50.72)	100 (89.39)	32.32 (30.45)
Hexaconazole	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)
Propiconazole	83.88 (66.30)	86.94 (68.78)	100 (89.39)	100 (89.39)	100 (89.39)	94.16 (80.65)
Chlorpyriphos	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)
Pendimethalin	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)	0.50 (4.05)
Mean	22.60 (21.62)	35.17 (30.77)	38.59 (34.82)	50.31 (44.51)	56.92 (50.38)	40.71 (36.42)
	Treatment (T)	Concentration (C)	T×C			
	CD at 5%	(0.08)	(0.06)	(0.19)		

*Mean of four replications. Figure in parenthesis indicate angular transformed values.

(DAS) in terms of disease incidence.

Efficacy of different botanicals under screen house conditions

For screen house experiments earthen pots were filled with sterilized sandy loam soil at the rate of 1 kg/pot. Four pots of each genotype (MH-4, MH-21, M-522, and HNG-10) with each treatment of powdered botanicals @ 20g/kg soil namely neem cake and mustard cake. Five seeds were planted in each pot and for each treatment four replications were maintained as CRD and each were inoculated with spore suspension (10^6/ml with the help of haemocytometer). Inoculated seeds sown in pots without botanicals served as control. Per cent disease incidence was recorded 30 DAS.

RESULTS AND DISCUSSION

Efficacy of different Fungicides and bio-agents against *A. niger in vitro*

Evaluation of fungicides/Pesticides

Efficacy of fungicides was tested *in vitro* under laboratory conditions for the per cent mycelial growth inhibition of *A. niger*. The data in Table 1 clearly revealed that propiconazole, carbendazim and carboxin completely inhibited the mycelial growth up to 100 per cent at 200, 500 and 1000 ppm concentration, respectively as comparable with 86.94, 88.05 and 59.96 per cent at their respective 100, 200 and 500 ppm. Captan and thiram were found very less effective as they inhibited 81.11 and 72.77 per cent of fungal growth, respectively at higher concentration of 1000 ppm. Hexaconazole (fungicide), pendimethalin (herbicide) and chlorpyriphos (insecticide) were also not found effective in inhibiting one per cent of the mycelial growth even at 1000 ppm.

Shekhawat *et al*. (1986) reported almost similar result as completely inhibition of the mycelial inhibition *in vitro* at 1500 ppm by carbendazim and carboxin. Captan and thiram were found very less effective as they inhibited 81.11 and 72.77 per cent of fungal growth, respectively at higher concentration of 1000 ppm, while fungicide hexaconazole, herbicide pendimethalin and insecticide chlorpyriphos have failed to inhibit the mycelial growth even at 1000 ppm. Gupta *et al.* (1974) found that *in vitro* studies growth of *A. niger* was completely inhibited at higher concentration of vitavax at 1500 ppm, captan and thiram at 2,000 ppm. No report for fungicide hexaconazole, herbicide pendimethalin and insecticide chlorpyriphos has been put forward as an effective pesticide against *A. niger*.

Evaluation of bio-agent *in vitro*

Three bio-agents namely *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluroscence* were tested for their inhibition of mycelial growth of *A. niger in vitro* (Table 2). *T. viride* showed maximum antifungal activity with 78.32 per cent inhibition of mycelial growth of *A. niger* followed by T. *harzianum* with 72.50 per cent inhibition of radial growth, while the bacterial bio-agent P. *fluroscence* inhibited 23.80 per cent of the mycelial growth of the test fungus over control.

These above results are in agreement with Gajera *et. al.* (2011) who demonstrated that twelve isolates of three *Trichoderma* strains *in vitro* against the collar rot, maximum inhibition of pathogen by *T. viride* followed by *T. harzianum*. Devi and Prasad (2009) also found similar result in which the per cent inhibition of the pathogen was maximum by *T. viride* followed by *T. harzianum* and very less effect of *Pseudomonas fluorescens* on inhibition of the pathogen *in vitro*. The parasitic behavior of *Trichoderma* was recorded to be of a necrotrophic or destructive type (Barnet and Binder, 1978; Transmo and Dennis, 1978).

Efficacy of promising seed treating fungicides and bio-agents on inhibition of *A. niger* under screen house conditions

Treated seeds with fungicides showed significantly less disease incidence for collar rot (Table 3). Fungicides were superior to bio-agents as fungicide treated seeds significantly controlled the collar rot disease. Maximum control was shown by propiconazole, controlling 87.50 per cent disease in genotype HNG-10 and 84.21 per cent in M-522. On an average, carbendazim reduced disease

Table 2. Effect of different bio-agents on percent mycelial inhibition of *A. niger in vitro*

Treatments	*Mycelial Inhibition (%)
Trichoderma viride	78.32(62.27)
Trichoderma harzianum	72.50(58.48)
Pseudomonas fluorescens	23.80(29.15)
C.D at 5%	4.93

*Mean of four replications, Figures in Parenthesis indicate angular transformation values.

Table 3. Efficacy of promising seed treating fungicides and bio- agents on inhibition of *A. niger* under screen house condition after 30 days of sowing

Treatments	Genotype MH-4		Genotype MH-21		Genotype M-522		Genotype HNG-10		Average
	*Disease Incidence (%)	*Disease control (%)	*Disease Incidence (%)	*Disease control (%)	*Disease Incidence (%)	*Disease control (%)	*Disease Incidence (%)	*Disease control (%)	Disease Control (%)
Captan (3g/kg)	21.42 (27.24)	64.71	25.00 (29.38)	53.33	28.57 (31.91)	57.89	17.85 (24.71)	68.76	61.17
Propiconazole (1ml / kg)	14.28 (22.19)	76.47	17.85 (24.71)	66.67	10.71 (17.65)	84.21	7.14 (13.12)	87.50	78.71
Carbendazim (2g/kg)	17.85 (24.71)	70.59	21.42 (27.24)	60.01	25.00 (29.38)	63.15	14.28 (22.19)	75.00	67.18
T. viride (20g/kg)	35.71 (36.58)	41.17	32.14 (34.05)	40.00	46.42 (42.83)	31.58	32.14 (34.05)	43.75	39.12
T. harzianum (20g/kg)	42.85 (40.78)	29.41	39.28 (38.63)	26.67	50.00 (44.97)	26.30	39.28 (38.63)	31.25	28.40
Control	60.71 (51.31)	_	53.57 (47.12)	_	67.85 (55.89)	_	57.14 (49.17)	_	_
	Variety(V)		Treatment(T)			V×T			
CD at 5%	(4.33)		(5.30)			(NA)			

*Mean of four replications, the values in parenthesis are angular transformation.

Table 4. Efficacy of different botanicals under screen house conditions after 30 days of sowing

Treatments	Genotype MH-4		Genotype MH-21		Genotype M-522		Genotype HNG-10		Average
	*Disease Incidence (%)	*Disease control (%)	*Disease Incidence (%)	*Disease control (%)	*Disease Incidence (%)	*Disease control (%)	*Disease Incidence (%)	*Disease control (%)	Disease Control (%)
Neem cake powder	50.00 (44.98)	29.15	52.38 (46.35)	26.74	47.36 (43.46)	30.78	46.15 (42.76)	32.53	29.80
Mustard cake powder l	53.84 (47.19)	23.71	56.25 (48.58)	21.24	52.94 (46.67)	22.62	52.63 (46.49)	23.97	22.88
Control	70.58 (57.66)	_	71.42 (57.74)	_	68.42 (55.82)	_	69.23 (56.37)	_	_
	Variety(V)		Treatment(T)			V×T			
CD at 5%	(NA)		(2.94)			(NA)			

Neem cake powder @ 20 g/kg soil, mustard cake powder @ 20 g/kg soil, *Mean of four replications. Figures in Parenthesis indicate angular transformation values.

incidence by 67.18 per cent and captan by 61.17 per cent. In case of Bio-agents, on an average, *T. viride* showed maximum control of the disease by 39.12 per cent followed by *T. harzianum* (28.40%).

Similar results were obtained by Karthikeyan (1996) the lowest disease incidence in which, he, reported carbendazim (2g/kg seed) was used as seed treatments as compared to disease incidence in control. Devi and Prasad (2009) also found same results, when applied captan as seed treatment @ 2g/kg seed under pot culture experiment.

Efficacy of different botanicals under screen house conditions

Among the botanicals, neem cake powder @ 20g/kg soil significantly controlled the disease up to 32.53 per cent followed by mustard cake powder @ 20g/kg soil which controlled the disease up to 23.97 per cent as comparison to untreated control (Table 4).

Organic amendments are recorded to favour native antagonists and suppress soil and seed borne diseases (Mayakrishnan, 1990; Alice, 1994). Similarly, Karthikeyan (1996) observed that the organic amendment with neem cake was highly effective in reducing disease incidence of collar rot of groundnut. Mustard cake was not found effective against collar rot disease in the present study.

CONCLUSION

It is concluded from the study, that the *Trichoderma viride* and propoiconazole showed their maximum antifungal activity *in vitro* and under screen house conditions and are needed to be tested under field conditions, so that they can be incorporated in integrated disease management strategies against this pathogen.

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