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EFFECT OF DIFFERENT FUNGICIDES, PLANT EXTRACTS ON INCIDENCE AND VARIETAL SCREENING AGAINST COLLAR ROT OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) CAUSED BY *ASPERGILLUS NIGER* VAN TIEGHAM

MANJU KUMARI, MAHABEER SINGH, SHAILESH GODIKA, SANGEETA CHOUDHARY AND JITENDRA SHARMA
Department of Plant Pathology, SKNCoA Jobner,
Sri Karan Narendra Agriculture University Jobner, Jaipur, Rajasthan - 332 029, INDIA
e-mail: Manjupawanda44@gmail.com

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***Corresponding author**

ABSTRACT
Groundnut is an economic important edible oilseed crop and suffers from seed, soil and foliar diseases. Collar rot of groundnut caused by *Aspergillus niger* is a disease of national importance and it is a limiting factor to groundnut cultivation. This disease has prevalent in almost all groundnut growing states in *kharif* season. To develop effective management strategies for this menacing disease using fungicides, plant extracts and verital screening were evaluated against *A. niger in vitro* and *in vivo* condition. The result revealed that companion (carbendazim + mancozeb) and Vitavax power at (50 to 250 ppm) followed by Bavistin (150 and 250 ppm) inhibit maximum fungal growth *in vitro* and in *in vivo* maximum disease control recorded in Companion (carbendazim + mancozeb) 71.83% followed by Bavistin 69.16% and Vitavax power 65.80% by seed treatment. In Plant-extracts, *in vitro*, Garlic clove extract at 10 and 15% concentration showed maximum growth inhibition of fungus followed by Neem and Datura leaves extract and *in vivo* maximum disease control was recorded in Garlic clove extract 40.60% followed by Neem leaves 34.78% at 15% concentration by seed soaking method. Fourteen varieties screened against collar rot, five varities observed moderately resistance, six varities found susceptible and three varities were highly susceptible.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a leguminous oilseed crop and India occupies the first position, both with regard to area and production of groundnut in the world. Groundnut is cultivated in more than 90 countries of the world (Virmani and Singh, 1985). Among them, Asia with (63.4%) area produces (71.7%) of world groundnut production. In Asia, leading producers of groundnut are India, China and Indonesia. The groundnut is grown over 18, 00,000 hectare of land throughout the world (Norden *et al.,* 1982). In India the total coverage area under this crop is about 5.5 million ha and production 9.5 million tones and average productivity 1723 kg/ha (Anonymous, 2013-14a). Major growing states of India are Gujarat, Andhra Pradesh, Rajasthan, Tamilnadu and Punjab. Among the states, Gujarat stand first in its production while Andhra Pradesh in area. Cultivation of groundnut crop in Rajasthan about 0.46 million hectares with annual production 0.90 million tones and productivity 1950 kg/ ha (Anonymous, 2013-14b). Groundnut is valuable for human food. Groundnut seeds contain protien (25.33%), carbohydrate (0.20%), fat (40.50%), fiber (3.4%) and ash (1.9%) and nearly half of the 13 essential vitamins and 20 essential minerals necessary for normal human growth and maintenance. Its roots contain numerous nodules which harbor of bacteria *Rhizobium* and fixes about 80-160kg N/ha per season (Alam *et al*., 1988). Among the biotic factors, diseases are major threat to groundnut production in many parts of the world. Groundnut suffers from a number of diseases which are caused by fungi, bacteria, viruses and nematodes. Collar rot of groundnut is one of serious diseases caused by *Aspergillus niger* and plays a major role in reducing groundnut yield. It is one of important seed and soil borne disease. This disease was first reported from India by Jain and Nenra (1952). The disease is expressing their symptoms in pre and post emergence phase. Many seed dressing fungicides are reported to be effective against collar rot of groundnut (Gangopadhyay, *et al.,* 1996, Karthikeyan, 1996). The loss due to this disease was reported 28 to 50% (Bakhetia, 1983). Looking to the losses due to this disease, field testing of combine molecules of fungicides was necessary as a seed treatment. The objective of the present study was to find out evaluate the various fungicides and Plant-extracts against collar rot disease *in vitro* and *in vivo*.

MATERIALS AND METHODS

In vitro and *in vivo* testing of fungicides

The field experiments were conducted consecutively during Kharif season 2015-16 in case house condition, of Department of Plant Pathology (Shri Karan Nrendra Agriculture University, Jobner, Jaipur (Rajasthan). The lab condition was concluded in CRD (Compete Randomized Design) with three replication. In laboratory condition the required quantities (50, 150 and

250 ppm) of six test fungicides were put in conical flask containing 100 ml molten PDA medium so as to get required concentration in ppm and poured in Petri-plates. A mycelial disc of 5 mm dia. of the pathogen taken from a 5 day old culture then placed at the center of the Petri- plate. A mycelial disc of the pathogen on PDA without adding any fungicide had served as control. Linear mycelial growth was recorded 7 days after incubation at 25 ± 1ºC, respectively. Per cent mycelial growth inhibition was calculated as per Vincent's (1947) formula:

$$\text{Percent mycelial growth inhibition} = \frac{C - T}{C} \times 100$$

Where, C = Diameter of the colony in control (average of both diagonals)

T = Diameter of the colony in treatment (average of both diagonals)

Further standard procedure was adopted on % growth inhibition of pathogen.

The field experiments for evaluate efficacy of fungicides against collar rot of groundnut incidence were conducted in Department of Plant Pathology, SKNAU Jobner, Jaipur in cage house condition. Local groundnut variety was used for study. Fungicides *viz*. Companion (carbendazim 12% + mancozeb 63%WP), Bavistin (carbendazim 50 WP), Vitavax power (Carboxin 37.5% + Thiram 37.5%WS), Steam (Captan 70% + Hexacanzole 5% WP), Raxil (Tebucanazole 2% DS) and Kavach (Chlorothalonil 96% W/W) 2g/kg seed were tested in pot culture by seed treatment. The field experiment was concluded in a randomized block design (RBD) with four replication. Prior to sowing, pots were sterilized with copper sulphate solution and filled with sterilized soil (soil: FYM = 3:1) sterilized at 1.045 kg/cm^2 for one hour for three consecutive days. These pots were inoculated with fungus inoculum multiplied on sorghum grain medium and shown 4 seeds/pot and without fungicide sown served as control. The timely observations were recorded up to 45 days. Per cent collar rot incidence was calculated by following formula:

$$\% \text{ Disease incidence} = \frac{\text{Number of rotted plants}}{\text{Total number of plants}} \times 100$$

***In vitro* and *in vivo* testing of plant extracts**

For testing efficacy of plant extracts of these plant parts were collected. Hundred gram leaves/cloves from plants was collected and washed 2-3 times with water and allowed to dry at room temperature (25 ± 1 ºC) for 15 minitue. Before extraction of leaves/cloves of each plant (100 g) were crushed separately with 100 ml sterilized water. The extracts were sterilized. Filtered through double-layered muslin cloth and tested as per the above technique at different concentrations (5, 10 and 15%). Poisoned food technique was used for the evaluation of antifungal potential (New, 1971). Per cent mycelial growth inhibition was calculated as per Vincent's (1947) formula as above.

In order to evaluate the effect of plant extracts on percent seedling emergence, disease incidence and disease control, the test seeds were soaked in 15 % plant extracts for 1 hrs. All 15% plant part extracts only was used for soaking seeds because these extracts exhibited antifungal properties in terms of percent inhibition of spore germination of test fungi (Sangvikar and Wadje, 2012). These seeds were sown in (2 × 2 m^2) micro-plot area. The pathogen multiplied on sorghum grains and mix in furrow at 8-10 cm depth @ 133g/2m row length to increase the disease pressure and calculate percent disease incidence as above mention formula.

Varietal screenings against collar rot of groundnut

Fourteen varities of groundnut received from Rajasthan Agriculture Research Institute, Durgapura, Jaipur and SKN college of Agriculture, Jobner, Jaipur were evaluated against collar rot of groundnut under plot condition during *Kharif* 2014-15. Fungus inoculum multiplied on sorghum grain medium was applied in furrow and sowing of different varities seeds. After sowing watering was done regularly as when required. The collar rot incidence was recorded up to up 45 days after sowing the basis on disease incidence the varieties were categorized according to their reaction against the disease as per criterion scale (0 to >30) (Free-0), (Resistance- 1to10), (Moderately resistance-11to20), (Susceptible-21to30) and (Highly susceptible >30).

RESULTS AND DISCUSSION

Results revealed that all the fungicides were capable of inhibiting the growth of the test fungus *in vitro* at different concentrations as compared to check. The data presented in (Table 1) Campanion and Vitavax power proved to be the most effective in inhibiting cent per cent growth of the test fungus at all the concentrations (150 and 250 ppm) followed by carbendazim (250 ppm). Kavach and Steam were not so effective at lower concentration. Although there was increase in growth inhibition with the increase in concentration. Because of these fungicides either inhibits the germination, growth and multiplication of the pathogen or is directly toxic. Our observations are in conformity to Nathawat and Mahandra partap (2014), evaluated effect of Carbendazim and Mancozeb (Companion), Carbendazim and Vitavax power against *A. niger*. Singh Gupta *et al*. (2014) found that carbendazim and mancozeb were most effective at higher doses against fungus *in vitro*. Singh and Singh (2006) found that carbendazim and mancozeb completely inhibited the fungal growth *in vitro* at higher concentrations. Among the combined fungicides, carbendazim + mancozeb proved to be the most effective in inhibiting (100%) the growth of fungus even at 250 ppm concentration. Singh *et al*. (2006) and Chakrabarty *et al*. (2013) also reported the higher yield of betel vine by the application of Carbedazim in comparison to untreated control.

The data presented in (Table 2) reveled that all treatments were significantly superior in *in vivo* over control. Companion was found most effective with (71.83%) disease control followed by Bavistin and Vitavax power with (69.16% and 65.80) disease control, respectively. Steam was least effective with (50.57%). It can be concluded that Companion as seed dresser was most effective in controlling collar rot of groundnut caused by *A. niger*. This finding collaborate with the finding of earlier workers Gangwar *et al*. (2014) reported that five fungicides used as seed treatments were evaluated against collar rot of groundnut and found that vitavax power (carboxin 37.50% + thiram 37.50%) @3.0g/kg seed was found

Table 1: Efficacy of fungicides against *Aspergillus niger* by poisoned food technique *(in vitro)*

Fungicide	Per cent Mycelium Growth Inhibition at Various Concentration (Ppm)			Average*
	50	150	250	
Campanion	91.66 (73.21)	95.34 (77.53)	100.00 (90.00)	95.67
Bavistin	85.00 (67.21)	92.31 (73.90)	100.00 (90.00)	92.44
Vitavax Power	90.00 (71.57)	95.30 (77.48)	100.00 (90.00)	95.10
Steam	85.71 (67.79)	89.57 (71.16)	90.85 (72.39)	88.71
Raxil	83.00 (65.65)	88.88 (70.52)	93.77 (75.55)	88.55
Kavach	81.00 (64.16)	85.00 (67.21)	92.50 (74.11)	86.17
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	92.44
	S. Em. +	CD (P = 0.05)		
Fungicide (F)	1.33	3.67		
Concentration (C)	2.03	5.61		
F × C	3.51	9.72		

*Average of three replications;Figures in parentheses are angular transformed values

Table 2: Efficacy of fungicides against collar rot incidence caused by *Aspergillus niger* *(in vivo)*

Treatment	Dose(gm)	Germination (%)	PDI*	Disease Control (%)
Companion	2	91.25 (72.79)	17.25 (24.35)	71.83
Bavistin	2	90.75 (72.29)	19.58 (26.26)	69.16
Vitavax	2	87.50 (69.30)	22.78 (28.51)	65.80
Steam	2	82.50 (65.27)	30.27 (33.38)	50.57
Raxil	2	81.25 (64.34)	24.34 (29.56)	60.26
Kavach	2	88.50 (70.18)	27.08 (31.36)	57.78
Control	-	74.00 (59.34)	61.25 (51.50)	0.00
S. Em.+		2.95	0.83	
CD (P = 0.05)		9.10	2.55	

Average of four replications , Figures in parentheses are angular transformed values;PDI = Percent disease incidence

Table 3: Efficacy of plant extracts against *Aspergillus niger* by poisoned food technique *(in vitro)*

Plant	Plant part used	Percent mycelium growth inhibition at various concentration			Average*
		5%	10%	15%	
Garlic	Clove	77.11 (61.42)	84.55 (66.85)	90.00 (71.57)	83.89
Tulsi	Leaf	61.80 (51.83)	64.90 (53.67)	68.53 (55.88)	65.08
Marigold	Leaf	50.40 (45.23)	52.80 (46.61)	58.53 (49.91)	53.91
Neem	Leaf	70.36 (57.01)	76.36 (60.91)	80.76 (63.98)	75.83
Datura	Leaf	65.48 (54.02)	68.83 (56.06)	71.48 (57.72)	68.60
Control		0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
		S. Em.+	CD (p = 0.05)		
Phyto extract (P)		0.88	2.43		
Concentration (C)		1.24	3.43		
P × C		2.15	5.95		

*Average of three replications;Figures in parentheses are angular transformed values

Table 4: Efficacy of plant extracts against collar rot incidence caused by *Aspergillus niger* (in vivo)

Treatment	Dose	Germination (%)	PDI*	Disease control (%)
Garlic	15%	81.25 (64.34)	37.03 (37.48)	40.60
Tulsi	15%	69.75 (56.63)	47.51 (43.57)	23.80
Marigold	15%	68.75 (56.01)	49.38 (44.64)	20.80
Neem	15%	78.00 (62.03)	40.66 (39.62)	34.78
Datura	15%	76.23 (60.82)	43.14 (41.06)	30.80
Control	15%	70.00 (56.79)	62.35 (52.15)	0.00
S. Em.±		0.87	0.68	
CD (p = 0.05)		2.68	2.09	

Average of four replications ;Figures in parentheses are angular transformed values;PDI = Percent disease incidence

minimum diseases incidence. Similar results were also obtained by Shivpuri *et al*. (2011). Mohapatra and Beher (2012) tested Bavistin, Mancozeb, Benlate, Captan and Thiram at @ 0.2% and 0.3% against *A. niger* reducing collar rot under pot conditions. Bavistin was found significantly effective followed by Benlate and Thiram. Tripathi (2015) also reported seed treatment and soil drenching with Hexaconozole (4.5 %) and Carboxin + Thiram (4.8 %) in comparison to control (21.7%) Sclerotial wilt..

In laboratory condition that the plant-extract (Table 3) of garlic

Table 5: Screening of groundnut varities against collar rot of groundnut

S.No.	Category	PDI	Varieties
1	Immune	0	None
2	Resistant	1-10	None
3	Moderately resistant	11-20	RG-425,CSNG-19-1,SNG-69,GG-21,RG-559-3
4	Susceptible	21-30	RG-578,RG-378,RG-582,M-13,Girnar-2,SNG-123
5	Highly susceptible	>30	RG-382,RG-510,Chitra

clove *(Alium sativum)* extract (77.11, 84.55 and 90.00 %) and neem leaf (*Azardirachta indica*) (70.36, 76.36 and 80.76 %) at 5, 10 and 15% concentration showed maximum growth inhibition over control. Extract of marigold and tulsi leaves was found least effective in inhibiting mycelial growth of *A. niger* over control. Similarly results have been observed by Nathawat and Partap (2014) while working with *A. niger in vitro*. Kumar *et al*. (2012) evaluated 17 plant extracts and 7 completely inhibited the mycelial growth *in vitro*. Extract of garlic clove and neem leaf proved to be most effective in inhibiting mycelial growth as well as in preventing spore formation. Wani and Kurucheve (2014) reported garlic clove extract (water) inhibited 100% mycelial growth *in vitro* of *A. niger* and *A. flavus*.

In field condition observed that the minimum disease incidence with garlic (37.03 %) followed by neem (40.66 %), over control (62.35 %) (Table 4). Maximum reduction in disease incidence was observed with garlic (40.60%) followed by neem (34.78%) over control. Tulsi (23.80 %) was found at par with marigold (20.80%). All plant extracts are able to reduce the disease incidence significantly over control. Similarly results have been observed by Patel *et al*. (2008), Yadav *et al*. (2007) and Mahapatra and Tewari (1994).

All the varieties of groundnut were found susceptible to collar rot except RG-425, CSNG-19-1, SNG-69, GG-21 and RG-559-3 were found moderately resistance (Table-5). None of variety was found immune or resistant to collar rot. Entries RG-578, RG-378, RG-582, M-13, Girnar-2 and SNG-123 were found susceptible and RG-382, RG-510 and Chitra were highly susceptible. Several workers have screened different varieties of groundnut for resistance against *A. niger* (Sakil Ahmed and Zaman, 2012) five varities of groundnut were evaluated for resistance to *A. niger, F. oxysporium and R. solani* at Punjab-Pakistan. Nathawat *et al*. (2014) evaluate five varities of groundnut against collar rot and only GG-2 was found tolerant.

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