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Effect of *Aspergillus niger* on seed germination and seedling vigor of groundnut genotypes

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Abstract: Effect of collar rot fungus, *Aspergillus niger* on seed germination and seedling vigour of groundnut genotypes was studied. *In vitro* experiment was conducted at the Department of Seed Science and Technology and in screen house of Department of Plant Pathology, CCS Haryana Agricultural University. Significant per cent reduction in plumule and radical length, fresh and dry weight including vigour index between inoculated and un-inoculated seeds with *A. niger* was observed. Similar reduction was also observed in per cent germination which ranged between 10.52 to 20.00 per cent in comparison to un-inoculated control. Plumule length of seedlings was significantly reduced *in vitro* conditions after inoculation with the pathogen, which ranged between 19.78 to 25.18 per cent. Similarly, the radical length was also significantly reduced in all genotypes within a range between 19.04 to 23.90 per cent in comparison to un-inoculated control. Fresh and dry weight of seedlings were also reduced by inoculation of seed in Petri plates by using blotter paper *in vitro* and the reduction in fresh weight ranged between 26.59 to 34.02 per cent, while the reduction in dry weight ranged between 26.19 to 37.93 per cent under seed inoculation technique on Petri plates in comparison to un-inoculated conditions.

Key words: *Aspergillus niger*, Seedling vigour, Vigour index

Introduction

Groundnut or peanut (*Arachis hypogaea* L.), is a very important oilseed crop of tropical and sub-tropical areas of the world, described in 1753 by Linnaeus (Pattee and Young, 1982). It is originated from Brazil in South America and was introduced in India by the Portuguese traders in the middle of sixteenth century. In India, groundnut occupies 35% of the total cropped area under oilseeds and accounts for 40% of total oilseeds production (Anonymous, 2014). On an average, groundnut seed contains 45% of oil and 26% 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% (Pande and Rao, 2000) and is more prevalent in soils with low moisture content and high temperature, approximately 30ºC (Kishore *et al*., 2007). The *A. niger* causing collar rot disease on groundnut seedlings was first reported by Jochem (1926). However, Jain and Nema, (1955) first reported the *Aspergillus* blight of groundnut caused by *A. niger* in India. This disease appears in two phases *viz*., pre-emergence and post- emergence phase. In the pre-emergence phase, the seed may rot in the soil or be covered with sooty black masses of spore on germination; the emerging hypocotyls are rapidly killed by these spores. In the post-emergence phase, circular light brown lesion appear initially on the cotyledons and as they advance the hypocotyls tissue or stem lesion becomes water-soaked and shows light brown discoloration. The seedlings then collapse and die due to the rotting of the succulent hypocotyls. It is prevalent in almost all groundnut growing states of India *viz*., Punjab, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Gujarat, Maharashtra, Rajasthan, Karnataka, Orissa and Haryana. The most of the groundnut cultivars are susceptible to this disease. Seedling vigour is one of the important quality parameters, which needs to be assessed with regard to seed germination and seedling viability tests to gain insight into the performance of a seed lot in the field or in storage. The present research work was undertaken to study the effect of *Aspergillus niger* on seed germination and seedling vigor of groundnut genotypes.

Materials and Methods

Laboratory studies: Ten healthy seeds for each replication of MH-4, MH-21, M-522 and HNG-10 genotypes of groundnut were surface sterilized and treated with 7 days old sporulating culture of *Aspergillus niger*. Seeds were dried for 72 hours at room temperature. The experiment was conducted in four replications and arranged as completely randomized design (CRD). Ten seeds of each genotype were placed at equal distance in each Petri plates by using the blotter seed health testing method (ISTA, 1999). In this method, replication of ten seeds in Petri plates containing three layers of water soaked blotters and incubated at room temperature. The un-inoculated surfaced sterilized seeds served as control for the experiment. After ten days of inoculation observations on per cent germination were recorded. The length of radicle and plumule, fresh and dry weight of ten days old healthy and fungal treated seedlings were recorded.

Screen house studies: Wheat grains were kept in polypropylene bags (500 g/bag) and plugged with non-absorbent cotton after putting

a plastic ring in the neck. Thereafter, bags were sterilized at 22 psi for 2 hours. The bits of pure cultured mycelium were inoculated in alternating layers of wheat and fungus mycelium and this set up was incubated for 10 days at 28±1 ºC to grow the fungus by utilizing the wheat grains to produce sufficient mycelium matter. Soil filled in earthen pots was mixed with inoculum in the screen house (30 ± 2ºC) to allow for maximum establishment. Ten healthy surface sterilized seeds of each genotype *viz*., MH-4, MH-21, M-522 and HNG-10 were sown at equidistance in the pots. The experiment was conducted in four replications and arranged as CRD. Seeds sown in un-inoculated pots to served as control. Observation was recorded on seed germination at 15 days after sowing and observations on shoot length and root length in centimeters, fresh and dry weight in grams and per cent collar rot incidence were recorded after 30 days of sowing.

Seedling Vigour Index was calculated with the help of germination per cent and seedling growth following ISTA guidelines (1999) as given below:

Vigour Index = Per cent Germination (%) x Seedling length (cm)

Results and Discussion

Percent germination and vigour of 10 days old groundnut seedling of different genotypes were significantly reduced by *A. niger* (Table 1). The per cent seed germination was reduced significantly in all the groundnut genotypes tested, however, maximum reduction of 20.0 per cent was observed in MH-4 followed by 18.2% in M-522 and 13.5% in MH-21, while minimum reduction of 10.5% was recorded in HNG-10 as compared to un-inoculated control. A significant decrease in plumule length and radical length were observed in all the genotypes, with maximum reduction of 25.2% (MH-4) and minimum of 19.8% (HNG-10) for plumule length and maximum reduction of 23.9% (MH-4) and minimum of 19.0% (HNG-10) as compared to un-inoculated control. Significant reductions in fresh and dry weights of different genotypes were also observed under *A. niger* inoculated conditions. Maximum reduction in fresh weight 34.0% (MH-4) and minimum reduction of 26.2% (HNG-10) while, for dry weight, maximum reduction of 37.9% (MH-4) and minimum of 26.2% (HNG-10) was observed as compared to un-inoculated controls. Maximum vigour index of 963.9 (HNG-10) and minimum of 656.1 (M-522) for inoculated seeds was observed as compared to maximum vigour index of 1335.7 (HNG-10) and minimum of 1052.7 (M-522) for un-inoculated. In terms of per cent reduction in vigour index of inoculated in comparison to un-inoculated, the maximum reduction of 39.54% (MH-4) and minimum of 27.83 (HNG-10) was recorded.

Percentage seed germination after 15 days of sowing and vigour of groundnut seedlings of different genotypes after 30 days of sowing was significantly reduced by *A. niger* inoculated soil conditions (Table 3). The data revealed that per cent germination was reduced significantly in all the groundnut genotypes tested, however, maximum reduction in seed germination 28.4% was observed in MH-4 followed by 27.6% in M-522 and 22.9% in MH-21, while minimum reduction of 19.4% in HNG-10 as compared to un-inoculated soil. During later stage of growth *i.e.* thirty days after sowing, the seedling vigour was significantly reduced. A significant decrease in shoot length was observed in all the genotypes, with maximum decrease of 26.9% in MH-4 and minimum of 23.1% in HNG-10 as compared to un-inoculated soil. Similarly, the root length was also significantly reduced in all the genotypes with maximum reduction of 23.6% in MH-4 and minimum of 20.2% in HNG-10 as compared to un-inoculated control. Reductions in shoot and root length have direct effect on reduction in seedling vigour index. Significant decreases in fresh and dry weights of different genotypes were also observed under *A. niger* inoculated soil.

Table-1: Effect of *A. niger* on seed germination and seedling vigour of 10 days old seedling by seed inoculation technique in Petri plates using blotter paper *in vitro*

Genotypes	*Percent seed germination			*Plumule length (cm)			*Radicle length (cm)			*Fresh weight (g)			*Dry weight (g)		
	I	UI	% Reduction	I	UI	% Reduction	I	UI	% Reduction	I	UI	% Reduction	I	UI	% Reduction
MH-4	70.00 (56.92)	87.50 (71.93)	20.00	3.95	5.28	25.18	5.92	7.78	23.90	0.64	0.97	34.02	0.18	0.29	37.93
MH-21	80.00 (63.78)	92.50 (76.00)	13.51	4.43	5.58	20.60	6.42	8.14	21.13	0.75	1.07	29.90	0.27	0.38	28.94
M-522	67.50 (55.41)	82.50 (65.81)	18.18	3.90	5.14	24.12	5.81	7.62	23.75	0.68	1.02	33.33	0.21	0.32	34.37
HNG-10	85.00 (70.27)	95.00 (80.46)	10.52	4.58	5.71	19.78	6.76	8.35	19.04	0.86	1.17	26.59	0.31	0.42	26.19
	CD at 5%		CD at 5%	CD at 5%			CD at 5%			CD at 5%					
Variety(V)	(9.61)			0.07			0.11			0.05			0.02		
Inoculation(I)	(6.79)			0.05			0.08			0.03			0.01		
V×I	(NA)			0.10			NA			NA			NA		

* = Mean of four replications. The values in parenthesis are angular transformation. I= Inoculated, UI= Un-inoculated

Table-2: Effect of *A.niger* on seedling vigour of 10 days old seedling by seed inoculation technique in Petri plates using blotter paper *in vitro*

Genotypes	Vigour Index-Inoculated			Vigour Index- Un-Inoculated			Reduction in
	*Germination (%)	*Seedling length (cm)	Vigour Index (% × cm)	*Germination (cm)	*Seedling length (cm)	Vigour Index (% ×cm)	Vigour Index (%)
MH-4	70.00	9.87	690.90	87.50	13.06	1142.75	39.54
MH-21	80.00	10.85	868.00	92.50	13.72	1269.10	31.60
M-522	67.50	9.71	656.10	82.50	12.76	1052.70	37.67
HNG-10	85.00	11.34	963.90	95.00	14.06	1335.70	27.83

* = Mean of four replications

Table-3: Effect of *A. niger* on seed germination of 15 days and seedling vigour of 30 days old seedling by soil inoculation technique under screen house conditions

Genotypes	*Percent seed germination (15 DAS)			*Shoot length (cm) (30 DAS)			*Root length (cm) (30 DAS)			*Fresh weight (g) (30 DAS)			*Dry weight (g) (30 DAS)			* % disease incidence (30 DAS)	
	I	UI	% Reduction	I	UI	% Reduction	I	UI	% Reduction	I	UI	% Reduction	I	UI	% Reduction	I	II
MH-4	55.00 (47.92)	80.00 (64.30)	28.40	16.38	22.42	26.94	8.75	11.46	23.64	4.78	7.28	34.34	0.96	1.48	35.13	45.45 (42.36)	0.50 (4.05)
MH-21	67.50 (55.41)	87.50 (71.93)	22.85	18.32	24.12	24.04	9.42	11.98	21.36	5.28	7.42	28.84	1.03	1.52	32.23	40.74 (39.64)	0.50 (4.05)
M-522	52.50 (46.42)	72.50 (58.58)	27.58	16.32	22.28	26.75	8.64	11.28	23.40	4.72	7.12	33.70	0.94	1.42	33.80	42.85 (40.83)	0.50 (4.05)
HNG-10	72.50 (58.58)	90.00 (73.96)	19.44	18.70	24.32	23.10	9.72	12.18	20.19	5.42	7.56	28.30	1.08	1.58	31.64	37.93 (37.99)	0.50 (4.05)
	CD at 5%			CD at 5%			CD at 5%			CD at 5%			CD at 5%			CD at 5%	
Variety(V)	(8.72)			0.18			0.12			0.11			0.04			(NA)	
Inoculation(I)	(6.16)			0.13			0.08			0.07			0.02			(1.32)	
V	(NA)			NA			NA			0.15			NA			(NA)	

* = Mean of four replication. The values in parenthesis are angular transformation. I= Inoculated, UI= Un-inoculated

Table 4: Effect of *A. niger* on seedling Vigour Index of 30 days old seedling by soil inoculation technique in Screen house

Genotypes	Vigour Index-Inoculated			Vigour Index- Un-Inoculated			Reduction in Vigour Index (%)
	*Germination (%)	*Seedling length (cm)	Vigour Index (% × cm)	*Germination (cm)	*Seedling length (cm)	Vigour Index (% ×cm)	
MH-4	55.00	25.13	1382.15	80.00	33.88	2710.40	49.00
MH-21	67.50	27.74	1872.45	87.50	36.10	3158.75	40.72
M-522	52.50	24.96	1310.40	72.50	33.56	2433.10	46.14
HNG-10	72.50	28.42	2060.45	90.00	36.50	3285.00	37.27

*Mean of four replications

Maximum reduction in fresh weight of 34.3% (MH-4) followed by 33.7% (M-522) and 28.8% (MH-21), with minimum reduction of 28.3% (HNG-10) was recorded as compared to un-inoculated soil. While, for dry weight, maximum reduction of 35.1per cent (MH-4) and minimum of 31.6per cent (HNG-10) was observed as compared to un-inoculated soil. Per cent disease incidence was also recorded; it was found that there was maximum disease incidence 45. 5% in MH-4 and minimum 37.9% in HNG-10 in *A. niger* inoculated soil conditions. Table 4 showed the vigour index of seedlings grown in soil inoculated with *A. niger* in comparison to un-inoculated soil. Maximum vigour index of 2060. 5 (HNG-10) and minimum of 1310.4 (M-522) for inoculated soil were observed as compared to maximum vigour index of 3285.0 (HNG-10) and minimum of 2433.1 (M-522) for un-inoculated soil. In terms of per cent reduction in vigour index of inoculated soil in comparison to un-inoculated soil, the maximum reduction of 49.0% (MH-4) and minimum of 37.3 (HNG-10) were recorded.

Similar observation were also reported by Shanker and Rao (1995) who mentioned that the effect of soaking of Green gram (*Vigna radiata* L.) seeds for six hours in culture filtrates of *A. niger* and found that there was reduction in seed germination. Similarly, Sadhu (2014) also reported that *A. niger* caused 30% reduction in seed germination of green gram. *A. niger* affected most adversely to seedling emergence (40%, control 90%), shoot length (5 cm, control 14 cm) and root length (5 cm, control 10 cm), respectively (Sadhu, 2014). Howlett (2006) reported toxins of seed borne fungi for inhibition of normal growth of seedlings in different crops.

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