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Management of Seedborne Mycoflora of Groundnut (*Arachis hypogaea* L.)

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Abstract

The present investigation was carried out on the "Studies of Seedborne Mycoflora of Groundnut (*Arachis hypogaea* L.)", with a view to assess seed borne mycoflora associated with the seeds of groundnut, their effect on seed germination and seedling vigour index, effects of fungicides and bioagents on seed borne mycoflora, seed germination and seedling vigour index. The externally seed borne pathogens of groundnut were detected by standard blotter test. Five seed borne fungi were found associated with seeds of groundnut externally, which were *Fusarium oxysporum, Macrophomina phaseolina, Penicillium spp., Aspergillus flavus* and *Aspergillus niger*. The internally seed borne fungi were detected by agar plate method and two fungi identified were Fusarium oxysporum and Macrophomina phaseolina. Among them Aspergillus flavus have highest incidence of 51.33 per cent. All the isolated pathogens were found pathogenic to groundnut resulting into reduction in seed germination and seedling vigour index. The seed borne mycoflora *viz*., *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Macrophomina phaseolina* and *Penicillium* spp were found more dominant and damaging. The lowest seed germination and seedling vigour index were noticed in the seeds inoculated with Aspergillus flavus which were 60.92 per cent and 908.75, respectively, as against 80.0 per cent and 1190.00 in control.

Key words : Bioagent, Seedborne mycoflora, Vigour index, Seed germination.

Groundnut belongs to the fabaceae family *Arachis hypogaea*, also known as the peanut and the goober nut. falls under the genus Arachis, which consists of 70 other species of flowering plants. The peanut is a legume crop, which means it belongs to the family Fabaceae and is primarily grown for human consumption or for livestock forage. It has been reported that South America was the place, from where cultivation of Groundnut originated and spread in Brazil, Southern Bolivia and North-western Argentina. Groundnut was introduced by the Portuguese from Brazil to West Africa and then to South-western India in the 16th century.

With annual all-season coverage of 54.2 lakh hectares, globally, India ranks first in Groundnut area under cultivation and is the second largest producer in the world with 101 lakh tonnes with productivity of 1863 kg per hectare in 2021-22. Gujarat is the largest producer of groundnuts in India. It is both grown during *Kharif* and *rabi* seasons. Apart from Gujarat, it is cultivated in West Bengal and Rajasthan.

The attack of various diseases at several growth stages, including seedborne infection, is one of the major limitations in groundnut production. During storage, fungi, bacteria and actinomycetes get associated with the seeds, inhibiting germination, causing seedling rot and causing infection in standing crops.

Material and methods

The seeds of groundnut varieties/genotypes will be collected from ARS, Niphad. The ISTA's standard blotter test as described by Neergaard (1979) was used for detection of external seed

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borne mycoflora of Groundnut. ISTA's standard agar plate method was used to detect internally seedborne mycoflora associated with seeds of the groundnut variety. (Neergard 1979). Kotchs postulate was used for proving the pathogenicity.

The effect of seed borne mycoflora of Groundnut on seed germination and seedling vigour index was studied by adopting the procedure of rolled towel paper described by ISTA (Anonymous, 1999). Total four hundred seeds of groundnut variety were inoculated with the individual pathogen by dipping the seeds in concentrated spore suspension (10^6 cfu ml^{-1}) for 12 hours. Then these inoculated seeds were dried in shade for 12 hours (Agarwal and Sinclair, 1993). On one towel paper, fifty seeds were placed and rolled carefully in order to avoid disturbances of seeds from their places. For each pathogen, eight towel papers were used, each with 50 seeds (for a total of 400 seeds). In a seed germinator, the wrapped towel papers were kept in a slanting position and incubated at 20-25°C with a RH over 85%. (Anonymous, 1999). After seven days, a count of normal seedlings was recorded. Germination was estimated in percentages for normal seedlings with full radical and plumule development. The root and shoot length (cm) of randomly selected ten normal seedlings from each towel paper were measured with the use of scale and seedling vigour index was calculated using formula which was given by Abdul-Baki and Anderson (1973).

SVI = [Mean root length (cm) + Mean shoot length (cm)] x Seed Gemination (%)

The per cent reduction in seed germination and seedling vigour index of seeds artificially inoculated with the pathogens over control was calculated. The data was statistically analysed (Panse and Sukhatme, 1985).

The seed germination and seedling vigour index was studied by adopting the procedure of rolled towel paper described by ISTA (Anonymous,1999)

The effect of fungicides and bioagents on artificially inoculated seeds was evaluated using the standard blotter test previously described. Under aseptic conditions, the seeds of the groundnut variety Mann were plated in petriplates according to the treatments. Seeds that had not been treated served as a control. In each treatment, total 400 seeds were used. With four replications, the experiment was performed using a Completely Randomized Design (CRD). For 7 seven days, the plates were incubated in an incubation room at 20 ± 2°C with an alternating cycle of 12 hours darkness and 12 hours light. On the eighth day, the number of seeds infected with mycoflora was observed using a sterio-binocular microscope, the per cent incidence of each mycoflora was recorded and the results were analysed statistically. The artificially inoculated seeds treated with fungicides, bioagents and botanicals were kept for germination by using between paper method as described earlier. Untreated seeds served as control. Seed germination, root and shoot length were recorded also seedling vigour index was calculated. Using the between paper method (Towel paper method), the seed germination of groundnut seeds treated with fungicides, bioagents and botanicals was studied. In the laboratory, the experiment was conducted in a Completely Randomized Design (CRD) with four replication. The data recorded in per cent to seed mycoflora and seed germination were converted into arc sin values. The final data of seed mycoflora, seed germination and seedling vigour index was statistically analysed by standard statistical method i.e., Analysis of variance. The standard error (SE) for above mentioned parameters were calculated and compared the treatments, critical difference (CD)

Table 1. Efficacy of seed treatment of fungicides on incidence of seedborne (artificially inoculated to seed) and their effect on seed germination and seedling vigour index of groundnut

Treatments	Incidence of seedborne mycoflora (%)					Reduction in incidence over control (%)					Seed germination (%)				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Carbendazim 50% WP @ 0.2 %	17.17 (24.47)	17.00 (24.35)	14.00 (21.97)	19.00 (25.59)	18.00 (25.14)	63.99	62.78	66.67	62.98	64.24	78.67 (62.49)	81.00 (64.15)	82.00 (64.89)*	76.67 (61.11)	79.67 (63.19)
Thiophanate methyl 70% WP @ 0.2%	22.33 (28.20)	22.33 (28.20)	20.33 (26.86)	24.50 (29.44)	24.33 (29.55)	53.15	51.10	51.59	52.92	51.65	74.33 (59.56)	78.67 (64.49)	79.67 (63.19)	73.00 (58.69)	76.00 (60.66
Carboxin 37.5 % + Thiram 37.5% @ 0.3%	13.50 (21.55)	15.50 (22.78)	8.66 (17.12)	14.00 (21.69)	14.67 (22.51)	71.68	67.16	79.37	73.37	70.86	82.00 (64.89)	84.00 (66.54)	94.33 (76.22)	79.33 (62.96)	83.00 (65.65)
Azoxystrobin 18.2% + Difenconazole 13.9% EC @ 0.1%	7.17 (15.52)	6.50 (14.77)	5.16 (13.13)	7.00 (15.15)	8.33 (16.77)	84.97	85.77	87.70	86.69	83.44	87.33 (69.15)	89.00 (70.63)	86.67 (68.58)	86.00 (68.02)	87.00 (68.86)
Picoxystrobin 6.78% + Tricycazole 20.33% EC @ 0.1%	13.00 (23.05)	16.00 (23.57)	14.00 (21.97)	15.00 (22.24)	16.33 (23.83)	72.73	64.97	66.67	72.08	67.55	80.33 (63.67)	81.00 (64.15)	84.67 (66.94)	78.67 (62.49)	82.33 (65.14)
Tebuconazole 50% + Tryfloxystrobin 25% WG @ 0.1%	5.30 (13.30)	4.33 (12.01)	4.66 (12.47)	4.00 (12.92)	5.67 (13.77)	88.88	90.67	88.89	90.26	88.74	91.67 (73.22)	91.67 (73.22)	88.00 (69.73)	88.67 (70.32)	89.33 (70.93)
Tebucanazole 45% WP @ 0.1%	19.00 (25.84)	18.33 (25.35)	19.00 (25.84)	23.00 (27.34)	20.00 (25.84)	60.90	59.86	54.76	57.79	60.26	74.33 (59.56)	78.67 (62.49)	80.67 (63.91)	75.00 (60.00)	79.00 (62.72)
Control	47.67 (43.66)	45.67 (42.51)	42.00 (40.39)	51.33 (46.14)	50.33 (45.19)	0.00	-	-	-	-	75.67 (52.33)	60.33 (52.73)	63.17 (52.63)	61.33 (47.86)	62.00 (51.94)
S. Em. ±		0.53	0.52	0.46	0.49	0.54					0.68	0.62	0.69	0.72	0.64
C.D. at 1%	1.60	1.58	1.39	1.49	1.63						2.07	1.88	2.88	2.19	1.95
C.V. (%)		4.99	4.99	4.98	4.38	4.77					1.5	1.33	1.47	1.62	1.40

Table 1. Contd.....

Treatments	Increase in seed germination over control (%)					Seedling Vigour Index (SVI)					Increase in SVI over control (%)				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Carbendazim 50% WP @ 0.2 %	30.00	27.90	23.44	25.01	28.49	1306.00	1292.67	1312.67	1232.67	1286.00	45.38	40.76	39.89	43.61	46.41
Thiophanate methyl 70 % WP @ 0.2%	21.87	24.22	16.67	19.03	22.58	1137.33	1157.33	1208.67	1097.33	1117.33	26.61	26.03	28.81	27.85	27.21
Carboxin 37.5 % + Thiram 37.5% @ 0.3%	36.25	32.64	30.73	29.37	33.87	1331.33	1351.33	1364.67	1291.67	1314.33	48.20	47.15	45.44	50.49	49.64
Azoxystrobin 18.2% + Difenconazole 13.9% EC @ 0.1%	46.25	40.53	33.33	40.23	40.32	1383.33	1403.33	1423.33	1343.33	1363.33	53.99	52.81	51.69	56.51	55.22
Picoxystrobin 6.78 % + Tricycazole 20.33% EC @ 0.1%	33.12	27.90	27.60	28.27	32.80	1301.33	1321.33	1341.33	1261.33	1281.33	44.86	43.88	42.95	46.95	45.88
Tebuconazole 50 % + Tryfloxystrobin 25% WG @ 0.1%	54.37	44.74	43.23	44.57	44.09	1433.33	1453.33	1473.33	1393.33	1413.33	59.56	58.26	57.02	62.33	60.91
Tebucanazole 45% WP @ 0.1 %	21.87	24.22	19.27	22.29	27.42	1207.00	1227.00	1247.00	1177.00	1187.00	34.36	33.61	32.90	37.13	35.14
Control		-	-		-	898.33	918.33	938.33	858.33	950.00	0.00	-	0.00		-
S. Em. ±						16.60	11.64	11.05	11.62	16.55					
C.D. at 1%						50.21	35.20	33.94	35.00	50.04					
C.V. (%)						2.30	1.59	1.50	1.66	2.33					

*Figures mentioned in parentheses indicates arc sin transformed values

A. *Fusarium oxysporum*
B. *Macrophomina phaseolina*
C. *Penicillium* spp.
D. *Aspergilus flavus*
E. *Aspergillus niger*

at 5 per cent level of significance was worked (Panse and Sukhatme, 1985).

Result and discussion

Detection of Externally Seedborne Mycoflora : The results showed that five fungi namely, *Fusarium oxysporum, Macrophomina phaseolina, Penicillium* sp., *Aspergillus niger* and *Aspergillus flavus* were found externally.

The results of internally seedborne mycoflora associated with groundnut seeds showed that two seedborne mycoflora *viz*., *F. oxysporum* and *M. phaseolina* were found internally associated groundnut seeds. (Adithya *et* al. 2017)

Efficacy of fungicides, bioagents and botanicals on incidence of seedborne *Fusarium oxysporum, Macrophomina phaseolina, Penicillium* sp., *Aspergillus niger* and *Aspergillus flavus* (artificially inoculated to seed) of groundnut

Among fungicides Tebuconazole 50% + Tryfloxystrobin 25 % WG @ 0.1 % which showed the higest reduction in the incidence of found most effective against *Aspergillus niger, Aspergillus flavus* and *Macrophomina phaseolina* was found to be the most effective among all fungicides. Where as Carboxin 37.5% + Thiram 37.5% DS @ 0.3% which showed the higest reduction in the incidence of and Fusarium oxysporum and Penicillium spp. over the inoculated control. Among bioagents Fungal consortium @ 10 gm kg^{-1} seed was found most effective with the highest reduction in incidence of *Fusarium oxysporum, Macrophomina phaseolina, Penicillium* sp., *Aspergillus niger* and *Aspergillus flavus* respectively The next best bioagents were Fungal consortium @ 5 gm kg^{-1} seed and *Psudomonas flurosense* + *Trichoderma harzianum* @ 5 gm kg^{-1} seed. (Bhoyar *et* al., 2014)

Efficacy of fungicides and bioagents on germination and seedling vigour index of groundnut seeds artificially inoculated with *Fusarium oxysporum, Macrophomina phaseolina, Penicillium* sp., *Aspergillus niger* and *Aspergillus flavus* (artificially inoculated to seed) of groundnut

Among fungicides Tebuconazole 50% + Tryfloxystrobin 25% WG @ 0.1% was found the most effective as it has shown maximum increase in seed germination, *Aspergillus niger, Aspergillus flavus* and *Macrophomina phaseolina* as well as increase in seedling vigour index respectively over the inoculated control, was found to be the most effective among all fungicides. whereas Carboxin 37.5% + Thiram 37.5 % DS @ 0.3% which showed shown maximum increase in seed germination of *Fusarium oxysporum* as well as increase in seedling vigour index over the inoculated control and *Penicillium* spp as well as increase in seedling vigour index over the control. Among bioagents Fungal consortium @ 10 gm kg^{-1} seed was found the most effective as it has shown maximum increase in seed germination of *Fusarium oxysporum, Macrophomina phaseolina, Penicillium* sp., *Aspergillus niger* and *Aspergillus flavus* as well as increase in seedling vigour index respectively. The next best bioagents were Fungal consortium @ 5 gm kg^{-1} seed and *Psudomonas flurosense* + *Trichoderma harzianum* @ 5 gm kg^{-1} seed. (Bhosale *et* al., 2016)

Conclusions

Five seedborne fungi *viz*., F*usarium oxysporum, Macrophomina phaseolina, Penicillium* spp., *Aspergillus flavus* and *Aspergillus niger* were detected externally on groundnut. Two internally seedborne fungi namely *F. oxysporum* and *Macrophomina phaseolina* were observed associated with seeds

Table 2. Efficacy of seed treatment of bioagent on incidence of seedborne (artificially inoculated to seed) and their effect on seed germination and seedling vigour index of groundnut

Treatments	Incidence of seedborne mycoflora (%)					Reduction in incidence over control (%)					Seed germination (%)				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Fungal consortium @ 5 gm kg^{-1} seed	22.33 (28.20)	17.67 (24.85)*	21.00 (27.27)	20.33 (18.42)	19.67 (26.32)	53.47	68.45	51.53	60.39	59.86	77.67 (62.02)	81.00 (64.15)*	80.00 (63.91)	79.00 (67.21)	78.00 (62.02)
Fungal consortium @ 10 gm kg^{-1} seed	22.67 (27.50)	16.67 (24.09)	19.33 (26.08)	20.00 (20.26)	19.33 (26.08)	55.56	70.24	55.38	61.04	60.53	76.00 (62.72)	82.00 (64.89)	80.66 (64.89)	80.00 (66.42)	79.00 (62.72)
Trichoderma viride @ 5 gm kg^{-1} seed	35.67 (33.83)	32.00 (34.44)	29.33 (32.79)	24.33 (23.57)	23.67 (29.10)	35.42	42.86	32.30	52.59	51.70	68.00 (55.75)	73.00 (58.69)	71.00 (57.41)	70.00 (64.16)	70.00 (56.78)
Trichoderma harzianum @ 5 gm kg^{-1} seed	33.00 (33.62)	30.33 (33.41)	27.66 (31.73)	24.33 (21.96)	22.67 (28.43)	36.11	45.83	36.15	52.59	53.74	70.33 (56.78)	76.00 (60.66)	73.00 (58.69)	72.00 (65.65)	70.67 (57.20)
Psudomonas flurosense + *Trichoderma viride* @ 5 gm kg^{-1} seed	31.33 (30.00)	20.33 (26.80)	25.66 (30.43)	22.67 (24.34)	21.67 (27.74)	47.92	63.69	40.76	55.84	55.78	71.00 (58.90)	78.00 (62.02)	73.00 (58.69)	73.00 (63.43)	72.67 (58.47)
Psudomonas flurosense + *Trichoderma harzianum* @ 5 gm kg^{-1} seed	28.00 (29.77)	18.67 (25.59)	23.00 (28.65)	21.33 (23.84)	20.67 (27.03)	48.61	66.67	46.92	58.44	57.82	72.33 (60.00)	80.00 (63.43)	77.00 (61.79)	76.33 (66.59)	74.33 (59.56)
Control	48.67 (43.85)	47.00 (43.28)	43.33 (41.16)	50.00 (48.44)	49.00 (44.42)	-	-	-	-	-	62.65 (52.73)	62.43 (52.19)	64.00 (53.13)	60.67 (46.14)	62.66 (52.33)
S. Em. ±	0.66	0.71	0.74	0.43	0.30						0.61	0.54	0.64	0.64	0.76
C.D. at 1%	2.04	3.00	2.28	1.33	1.15						1.89	2.29	1.96	1.96	2.32
C.V. (%)	3.98	4.73	4.77	2.87	2.59						1.47	1.24	1.49	1.52	1.83

Table 2. Contd....

Treatments	Increase in seed germination over control (%)					Seedling Vigour Index (SVI)					Increase in SVI over control (%)				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Fungal consortium @ 5 gm kg^{-1} seed	23.16	29.75	26.04	30.23	25.81	1363.00	1433.00	1403.00	1363.00	1346.33	42.38	39.49	40.68	42.38	43.63
Fungal consortium @ 10 gm kg^{-1} seed	24.74	31.35	28.13	31.88	27.42	1371.00	1441.00	1411.00	1371.80	1347.67	43.21	40.27	41.48	43.12	43.78
Trichoderma viride @ 5 gm kg^{-1} seed	7.90	16.93	10.94	15.40	12.90	1337.00	1407.00	1377.00	1337.50	1316.67	39.66	36.96	38.07	39.66	40.47
Trichoderma harzianum @ 5 gm kg^{-1} seed	10.53	21.74	14.06	18.69	13.98	1340.00	1410.00	1380.00	1340.00	1320.00	39.97	37.25	38.37	39.97	40.83
Psudomonas flurosense + *Trichoderma viride* @ 5 gm kg^{-1} seed	15.80	24.94	14.06	20.34	17.20	1345.00	1415.00	1385.00	1345.00	1324.67	40.49	37.74	38.87	40.49	41.32
Psudomonas flurosense + *Trichoderma harzianum* @ 5 gm kg^{-1} seed	18.43	28.14	21.35	25.84	19.89	1350.00	1420.33	1390.00	1350.00	1330.00	41.02	38.22	39.37	41.02	41.89
Control	-	-	-	-	-	957.33	1027.33	997.33	957.33	937.33	-	-	-	-	-
S. Em. ±		-			-	20.20	20.20	20.20	20.19	20.25					-
C.D. at 1%						61.86	85.04	61.86	61.86	62.04					
C.V. (%)						2.70	2.56	2.62	2.07	2.75					

*Figures mentioned in parentheses indicates arc sin transformed values

A. *Fusarium oxysporum*
B. *Macrophomina phaseolina*
C. *Penicillium* spp.
D. *Aspergilus flavus*
E. *Aspergillus niger*

of groundnut. All the isolated pathogens were found pathogenic to seeds of groundnut resulting into reduction in seed germination and seedling vigour index. The seedborne mycoflora *viz*., *Aspergillus flavus, Aspergillus niger* and *Fusarium oxysporum* were found more damaging. Among all the treatments of fungicide, bioagents and botanicals fungicidal seed treatments were found significantly superior in reducing seedborne mycoflora, increasing seed germination and seedling vigour index. Among fungicides Tebuconazole 50 % + Tryfloxystrobin 25% WG @ 0.1 % were found most effective against *Aspergillus niger, Aspergillus flavus* and *Macrophomina phaseolina* whereas Carboxin 37.5% + Thiram 37.5 % DS @ 0.3 % were found most effective against *Fusarium oxysporum* and *Penicillum* spp. Among all the bioagents seed treatment, Fungal consortium @ 10 gm kg^{-1} seed recorded lowest seed mycoflora, highest increase in seed germination and seedling vigour index in artificially inoculated seed with all seedborne *Fusarium oxysporum*, *Macrophomina phaseolina, Penicillium* spp., *Aspergillus flavus* and *Aspergillus niger.*

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