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Effect of seed mycoflora on seed health in respect to seed germination and seedling vigour in groundnut cultivars

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Abstract
Groundnut or peanut (*Arachis hypogaea* L.) is a crop of global importance, also known as "King of oilseeds". It is widely grown in the tropical and subtropical regions of the world. Quality of seeds play a very important role for the production of healthy crop. A seed-borne pathogen present externally or internally or associated with the seed as contaminant may caused seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as caused biochemical changes in infected seeds. Over all, six fungal species belonging to three genera *viz*., *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Rhizopus* sp., *Aspergillus fumigatus* and *Aspergillus terreus* were detected as seed mycoflora in groundnut by agar plate and blotter paper method. *Aspergillus flavus* Link and *Aspergillus niger* van Tieghem were found dominant seed mycoflora in groundnut in all ten cultivars both in agar plate and blotter paper method. The six different isolates detected from groundnut seed samples were identified based on morphological characters. However, among these, two unknown isolates were sent to Indian Type Culture Collection, Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi for identification and it was identified as *Aspergillus fumigatus* Fresenius (ITCC No. 10,442.17) and *Aspergillus terreus* Thom (ITCC No. 10,441.17). The seed germination, seedling length and seedling vigour index (SVI) was drastically reduced by all detected seed mycoflora in all tested groundnut cultivars. *Aspergillus flavus* and *A. niger* caused maximum reduction in germination per cent, seedling length and SVI in all ten cultivars. *Aspergillus flavus* showed highest Per cent Discolouration Intensity (PDI) in cultivars *viz*., GG 8 (77.75%), GG 2 (77.25%) and GG 11 (75%).

Keywords: groundnut, seed mycoflora, agar plate method, blotter paper method, paper towel method, seed vigour index

Introduction
Groundnut or peanut (*Arachis hypogaea* L.) is a crop of global importance, also known as "King of oilseeds". It is widely grown in the tropical and subtropical regions of the world. The crop is believed to have originated in Brazil. It is an important crop to both small holder and large commercial producers. It is classified both as a grain legume and an oilseed crop. It plays a major role in bridging the vegetable oil deficit in the country. It contains 48-50 per cent oil, 26-28 per cent protein and is rich in dietary fibers, minerals and vitamins (Rani, 2014) [15]. Groundnut belongs to the botanical family: Fabaceae (Leguminosae). The specific name *hypogaea* means "under the earth" because groundnut pods develop underground a feature known as geocarpy. Like most other legumes, groundnut harbour symbiotic nitrogen-fixing bacteria in root nodules. This capacity to fix nitrogen implies groundnut require less nitrogen-containing fertilizer and improve soil fertility, thus are valuable in crop rotations. Total production of groundnut in the world is reported to be 43.9 million tons (Anon., 2014) [5]. It is mainly grown in China, India, Nigeria, USA, Sudan, Burma, Indonesia, Argentina, Tanzania and Senegal. India is the second largest producer of groundnut in the world, which is grown in an area of 4.8 million hectares with an annual production of 74.20 lakh tons (Anon., 2015) [6]. It is mainly grown in the states of Andhra Pradesh, Gujarat, Karnataka, Maharashtra, Madhya Pradesh, Tamil Nadu, Rajasthan and Uttar Pradesh among which, Gujarat leads in area and production. Annually, 40.65 lakh tons of groundnut are exported mainly to South-East Asian countries, namely, Indonesia, Malaysia, Vietnam and also to neighbouring countries like Pakistan, Sri Lanka and Nepal (Anon., 2013) [4].

In Gujarat, it is mainly grown in districts *viz.*, Junagadh, Jamnagar, Rajkot, Porbandar, Amreli, Bhavnagar, Kutch, Sabarkantha and Banaskantha, *etc*. with a total area of 14.01 lakh hectares

with annual production of 30.18 lakh tons (Anon., 2015) [6]. The aflatoxin producing molds exist throughout the groundnut growing areas. It is also reported that *A. flavus* is the important mycotoxin producer and produce aflatoxin B_1, B_2, G_1 and G_2, which are hepatocarcinogenic. Mycotoxin can cause severe damage to the liver, kidneys and nervous system of human being even at low dosages (Rodricks, 1976) [16]. A seed-borne pathogen present externally or internally or associated with the seed as contaminant may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection (Halt, 1994) [8]. Fungi like *Aspergillus niger*, *Aspergillus flavus*, *Alternaria dianthicola*, *Curvularia lunata*, *Curvularia pallescens*, *Fusarium oxysporum*, *Fusarium equiseti*, *Macrophomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum* and *Penicillium chrysogenum* causes discoloration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification to oilseeds (Chavan and Kakde, 2008) [7]. Adiver and Kumar (2015) [3] reported that seed mycoflora reduced seed quality of groundnut. The infection lead to the reduction in sugars (reducing, non-reducing and total sugars), proteins, oil content and seed germination.

Materials and Methods

Seed Inoculation with Seed Mycoflora

Groundnut seeds of ten cultivars were artificially inoculated with dominant seed mycoflora separately. Seeds moistened by sterilized water were mixed thoroughly with 10 days old respective fungal culture growth obtained on PDA at 25±2 ˚C. Such treated seeds were kept in Petri plates for overnight at 25±2 ˚C, and then these seeds were used for seed germination, seedling vigour index study and assessment of seedling abnormalities.

Effect on Seed Germinability

Effect of seed mycoflora on seed germination was tested by paper towel method (Fig. 1) (Sahu and Agrawal, 2003) [18]. One sheet of germination paper was wetted by distilled water. Twenty-five seeds of respective groundnut cultivars inoculated with respective seed mycoflora were placed on first sheet evenly. Second sheet of germination paper was placed on first sheet. Second sheet was wetted carefully. Both sheets were rolled along with wax coated paper. The rolled papers were incubated in seed germinator at 25˚C for 10 days. At the end of incubation, rolled towel papers were carefully opened. Germinated and ungerminated seeds were counted treatment-wise and cultivar-wise. Healthy seeds without inoculation of seed mycoflora were considered as control. Four repetitions of each of 100 seeds were maintained for each of the treatments. Seedling length of germinated seeds were recorded.

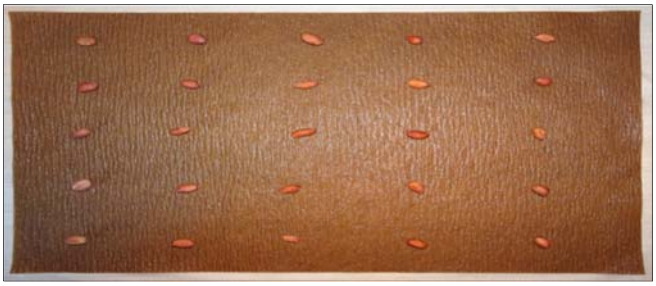

Fig 1: Seed germination test by Paper towel method

Seedling Vigour Index

The seedling vigour index (SVI) was computed after 10 days of incubation by adopting the method suggested by Abdul Baki and Anderson (1973) and expressed as whole number.
SVI= Germination percentage X Seedling length (cm)

Assessment of Seedlings by Symptoms Grade

Ten days after the incubation of inoculated seeds of respective cultivars, by paper towel method, each of the developing seedling were critically observed and if required with the help of magnifying hand lens for seedling discoloration due to seed mycoflora infection on developing seedlings. Seedlings were categorized into 0-4 rating scale. Four repetitions each of 100 seeds were evaluated for each of the treatment.

Rating Scale Description

0 Healthy seedling (no visible symptoms)
1 Discoloration of 1-10% part of seedling
2 Discoloration of 11-25% part of seedling
3 Discoloration of 26-50% part of seedling
4 Discoloration of > 50% part of seedling

The following equation was used to calculate the Per cent Discolouration Intensity (PDI) given by Kotastathane and Agarwal (1976) [10].

$$PDI = \frac{\text{Sum of all rating}}{\text{No. of seedling examined} \times \text{Maximum rating scale}} \times 100$$

Results and Discussion

Effect of Seed Mycoflora on Seed Germination

Result on seed germination as influenced by seed mycoflora presented in Table 1 revealed significant differences. All the test fungi showed significantly pronounced inhibitory effects on seed germination of groundnut cultivars as evident from significantly reduced germination percentage as compared to control treatment. *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Rhizopus* sp, *Aspergillus fumigatus*, *Aspergillus terreus* and control (untreated) showed germination in the mean range of 56.17, 61.00, 66.00, 68.70, 71.15, 73.15 and 89.45 per cent, respectively. Significantly lowest seed germination was found in GG 4 (63.96%) which was followed by GG 2 (66.28%) and GG 7 (67.67%) cultivars. Maximum germination per cent reduction recorded in *Aspergillus niger* (56.17%) inoculated seeds, which was followed by *Aspergillus flavus* (61%) and *Fusarium oxysporum* (66%). Nargund *et al*. (2003) [12] reported that *Aspergillus flavus*, *A. niger*, *Rhizoctonia* sp., *Fusarium* sp. and *Sclerotium rolfsii* reduced germination percentage and vigour index in all genotypes of groundnut. Raj *et al*. (2002) [13] identified the species of *Aspergillus*, *Alternaria*, *Rhizoctonia*, *Fusarium*, *Phoma* and *Chaetomium* were

affecting germination and emergence of soybean seeds. Abbas *et al.* (2013) [1] studied effect of seed mycoflora on the quality of three genotypes of groundnut *viz.*, Chandra, Indori and Rajasthani and reported that infection of *A. flavus* caused both quantitative and qualitative damage to the seeds and it resulted in reduction of germination percentage and seedling vigour index. The present results are also in agreement with above workers.

Table 1: Seed germination of groundnut cultivars as influenced by seed mycoflora

Cultivars	Germination (%)							
	Aspergillus niger	*Aspergillus Flavus*	*Fusarium oxysporum*	*Rhizopus* sp.	*Aspergillus fumigatus*	*Aspergillus terreus*	Control	Mean (C)
GG 2	46.50	51.00	61.75	70.00	73.50	75.00	86.25	66.28
GG 3	59.75	63.75	67.75	69.75	72.75	74.00	88.75	70.92
GG 4	50.50	49.00	54.75	66.75	67.00	70.50	89.25	63.96
GG 5	56.50	64.00	74.75	69.50	72.25	73.00	90.50	71.50
GG 7	56.50	57.75	62.75	65.00	73.00	71.00	87.75	67.67
GG 8	63.00	65.50	67.00	68.25	67.25	72.50	92.75	70.89
GAUG 10	61.50	70.50	67.75	63.25	64.00	74.25	89.75	70.14
GG 11	50.75	56.75	62.25	69.50	72.50	73.25	90.25	67.89
GG 13	55.00	62.75	67.50	69.75	73.00	72.25	88.00	69.75
GG 20	61.75	69.00	73.75	75.25	76.25	75.75	91.25	74.71
Mean (F)	56.17	61.00	66.00	68.70	71.15	73.15	89.45	-

	C	F	C X F
S.Em. ±	0.72	0.60	1.92
C.D. at 5%	2.03	1.69	5.37
C.V%	5.55		

Note: C = Cultivars, F= Fungi

Effect of Seed Mycoflora on Seedling Length

Result on assessment of seedling length of groundnut cultivars as influenced by seed mycoflora carried out by paper towel method is presented in Table 2. Result showed that all test fungi significantly reduced seedling length as compared to control. *Aspergillus niger* showed maximum detrimental effect thereby recorded minimum seedling length in all cultivars *viz*., GG 2 (5.32 cm), GG 3 (3.87 cm), GG 4 (4.47 cm), GG 5 (3.48 cm), GG 7 (6.32 cm), GG 8 (4.11 cm), GAUG 10 (6.66 cm), GG 11 (4.45 cm), GG 13 (4.30 cm) and GG 20 (4.26 cm).

Aspergillus terreus showed minimum inhibitory effect in seedling length in GAUG 10 (9.47 cm), GG 2 (9.07 cm), GG 20 (9.07 cm) and GG20 (9.17 cm) with *A. fumigatus* as compared to other cultivars. Overall, *Aspergillus niger*, *A. flavus*, *F. oxysporum*, *Rhizopus* sp., *A. fumigatus*, *A. terreus* and control revealed the seedling length in all cultivars between 3.48 to 6.66, 4.46 to 6.22, 5.68 to 7.35, 4.42 to 8.07, 6.10 to 9.17, 6.03 to 9.47 and 9.17 to 16.40 cm, respectively. Tripathi (1974) [19] studied seed mycoflora of cereals and reported that, culture filtrate of *Aspergillus flavus* was inhibitory to root and shoot growth. Sadhu (2014) [17] studied seed borne fungi and their effect on seed health of green gram and observed that *Aspergillus niger* and *Drechslera tetramera* affected most adversely to seedling emergence, shoot length and root length. Kandhare (2015) [9] studied effect of fungal metabolites on seed health of pulses and showed that mycotoxins obtained from all common and dominant seed-borne fungi affected adversely in seed germination, shoot and root length. The present findings are tallied with the similar studies carried out by above scientists.

Table 2: Seedling length of groundnut cultivars as influenced by seed mycoflora

Cultivars	Seedling length (cm)							
	Aspergillus niger	*Aspergillus flavus*	*Fusarium oxysporum*	*Rhizopus* sp.	*Aspergillus fumigatus*	*Aspergillus terreus*	Control	Mean (C)
GG 2	5.32	6.22	7.00	8.05	8.55	9.07	13.52	8.25
GG 3	3.87	5.12	6.36	6.45	7.22	6.15	11.02	6.60
GG 4	4.47	5.97	6.76	5.55	7.30	7.87	10.52	6.92
GG 5	3.48	4.46	5.68	4.42	6.10	7.22	14.37	6.53
GG 7	6.32	5.33	5.98	4.92	6.52	6.03	9.17	6.32
GG 8	4.11	5.92	6.76	5.88	6.92	7.40	14.87	7.41
GAUG 10	6.66	7.15	7.35	6.50	8.82	9.47	16.40	8.90
GG 11	4.45	5.58	5.98	6.57	7.77	6.06	12.85	7.04
GG 13	4.30	5.25	6.77	8.07	8.60	7.77	13.55	7.76
GG 20	4.26	6.15	6.61	8.07	9.17	9.07	16.02	8.48
Mean (F)	4.72	5.71	6.52	6.45	7.70	7.61	13.23	-

	C	F	C X F
S.Em. ±	0.13	0.11	0.35
C.D. at 5%	0.37	0.31	1.00
C.V%	9.68		

Note: C = Cultivars, F= Fungi

Effect of Seed Mycoflora on Seedling Vigour Index

Result on seedling vigour index (SVI) as influenced by individual seed mycoflora are presented in Table 3. Seedling vigour index worked out by multiplying the seedling length with germination per cent revealed significant differences. Each of seed mycoflora significantly reduced SVI of all cultivars over the control. In respect of mean SVI of ten cultivars, *Aspergillus niger* showed the maximum detrimental effect thereby recorded minimum SVI (265.19) followed by *Aspergillus flavus* (349.16) and *Fusarium oxysporum* (430.06), while *Aspergillus terreus* (557.73) revealed the lowest adverse effect on SVI among six seed mycoflora evaluated. The remaining seed mycoflora *viz.*, *Rhizopus* sp. and *Aspergillus fumigatus* showed SVI in between 307.19 to

699.21. Seedling vigour index of cultivar GG 2, GG 3, GG 4, GG 5, GG 7, GG 8, GAUG 10, GG 11, GG 13 and GG 20 as influenced by various seed mycoflora ranged between 247.38 to 680.25, 231.23 to 525.25, 225.73 to 554.83, 196.62 to 527.06, 307.80 to 475.96, 258.93 to 536.50, 409.59 to 703.14, 225.83 to 563.32, 236.50 to 627.80 and 263.05 to 699.21, respectively. Cultivar GG 5 revealed lowest SVI (196.62) due to *Aspergillus niger* treatment, which was followed by GG 4 (225.73) and GG 11 (225.83). Nargund *et al.* (2003) [12] reported that *Aspergillus flavus*, *A. niger*, *Rhizoctonia* sp., *Fusarium* sp. and *Sclerotium rolfsii* reduced germination percentage and vigour index in all genotypes of groundnut seeds. Krishnappa *et al*. (2003) [11] reported that groundnut pods stored in gunny bag had recorded maximum infection ranged between 16 and 18 per cent of *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp. and *Penicillium* sp. and caused reduction in germination and vigour index. The present finding are also in agreement with above scientists.

Table 3: Seedling vigour index of groundnut cultivars as influenced by seed mycoflora

Cultivars	Seedling vigour index							
	Aspergillus niger	*Aspergillus flavus*	*Fusarium oxysporum*	*Rhizopus* sp.	*Aspergillus fumigatus*	*Aspergillus terreus*	Control	Mean (C)
GG 2	247.38	317.22	432.25	563.50	628.42	680.25	1166.10	576.45
GG 3	231.23	326.40	430.89	449.88	525.25	455.10	978.02	485.25
GG 4	225.73	292.53	370.11	370.46	489.10	554.83	938.91	463.09
GG 5	196.62	285.44	424.58	307.19	440.72	527.06	1300.48	497.44
GG 7	357.08	307.80	375.24	319.80	475.96	428.13	804.66	438.38
GG 8	258.93	387.76	452.92	401.31	465.37	536.50	1379.19	554.57
GAUG 10	409.59	504.07	497.96	411.12	564.48	703.14	1471.90	651.75
GG 11	225.83	316.64	372.25	456.61	563.32	443.89	1159.71	505.46
GG 13	236.50	329.43	456.97	562.88	627.80	561.38	1192.40	566.76
GG 20	263.05	424.35	487.48	607.26	699.21	687.05	1461.82	661.46
Mean (F)	265.19	349.16	430.06	445.00	547.96	557.73	1185.32	-
				C	F		C X F	
S.Em. ±				5.69	4.76		15.08	
C.D. at 5%				15.89	13.29		42.04	
C.V%				5.58				

Note: C = Cultivars, F = Fungi

Per cent Discolouration Intensity of Groundnut Seedlings

Result on per cent discoloration intensity (PDI) as influenced by individual seed mycoflora are presented in Table 4. Result showed that significant differences in per cent discoloration intensity in all groundnut cultivars. *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Rhizopus* sp., *Aspergillus fumigatus* and *Aspergillus terreus* showed PDI between 51.25 to 74.25, 62.75 to 77.75, 28.75 to 65.50, 37 to 57.50, 37.25 to 53.00 and 26.25 to 39.75%, respectively. *Aspergillus flavus* showed highest PDI in cultivars *viz*., GG 8 (77.75%), GG 2 (77.25%) and GG 11 (75%). *Aspergillus terreus* showed significantly lowest discoloration intensity in cultivar GG 20 (26.25%) which was at par with GG 5 (26.50%) and followed by GG 3 (29%) and GAUG 10 (29%). In respect of mean PDI showing overall effects on seedling irrespective of groundnut cultivars, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Rhizopus* sp., *Aspergillus fumigatus*, *Aspergillus terreus* showed PDI in between 33.42 to 69.55% in descending order. Rakholiya *et al.* (2012) [14] reported that collar rot of groundnut expressed both during pre-and post-emergence phases and infected seeds became black and did not germinated. Chavan and Kakde (2008) [7] reported that groundnut seeds were highly susceptible to diseases, as they served as a source of stored nutrients for fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Alternaria dianthicola*, *Curvularia lunata*, *Curvularia pallescens*, *Fusarium oxysporum*, *Fusarium equiseti*, *Macrophomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum* and *Penicillium chrysogenum* causes discoloration, rotting, shrinking, seed necrosis, loss in germination capacity. Thus, the present results are in agreement with the results obtained by earlier workers.

Table 4: Discolouration intensity of groundnut cultivars influenced by seed mycoflora

Cultivars	Discolouration intensity (%)							
	Aspergillus niger	*Aspergillus flavus*	*Fusarium oxysporum*	*Rhizopus* sp.	*Aspergillus fumigatus*	*Aspergillus terreus*	Control	Mean (C)
GG 2	72.25	77.25	54.75	46.25	42.00	35.25	6.5	47.75
GG 3	74.00	70.50	64.50	53.75	38.25	29.00	5.5	47.92
GG 4	71.50	69.75	63.75	53.75	53.00	33.25	5.75	50.10
GG 5	71.00	67.75	49.25	42.75	46.75	26.50	4.75	44.10
GG 7	51.25	62.75	28.75	46.50	37.25	39.0	7.25	38.96
GG 8	73.75	77.75	65.50	54.75	49.50	38.25	6.0	52.21
GAUG 10	68.25	54.75	45.25	46.50	39.75	29.00	4.25	41.10
GG 11	67.25	75.00	62.50	37.0	52.0	38.0	5.75	48.21
GG 13	72.00	66.50	46.25	57.50	48.50	39.75	7.50	48.28
GG 20	74.25	66.75	52.75	41.50	45.25	26.25	5.50	44.60
Mean (F)	69.55	68.87	53.32	48.02	45.22	33.42	5.87	-
				C	F		C X F	
S.Em. ±				0.51	0.43		1.36	
C.D. at 5%				1.44	1.20		3.81	
C.V.%				5.90				

Note: C = Cultivars, F=Fungi

Conclusion

All the test fungi showed significantly pronounced inhibitory effects on seed germination of groundnut cultivars as evident from significantly reduced germination percentage as compared to control treatment. Maximum germination per cent reduction recorded in *A. niger* (56.17%) inoculated

seeds, which was followed by *A. flavus* (61%) and *Fusarium oxysporum* (66%). Significantly lowest seed germination found in GG 4 (63.96%) which was followed by GG 2 (66.28%) and GG 7 (67.67%) cultivars. Similar detrimental effect on seedling length of all ten cultivars was observed due to seed mycoflora. *Aspergillus niger* showed maximum detrimental effect thereby recorded minimum seedling length in all cultivars. *Aspergillus terreus* and showed minimum inhibitory effect in seedling length in GAUG 10 (9.47 cm), GG 2 (9.07 cm), GG 20 (9.07 cm) and GG20 (9.17 cm) with *A. fumigates* as compared to other cultivars. Seed Mycoflora also caused the adverse effect on the seedling vigour index in all the ten cultivars with respect to mean SVI of ten cultivars. *Aspergillus niger* showed the maximum detrimental effect thereby recorded minimum SVI (265.19) followed by *A. flavus* (349.16) and *Fusarium oxysporum* (430.06) while, *A. terreus* (557.73) revealed the lowest adverse effect on SVI among six seed mycoflora evaluated. Seed mycoflora showed significant differences in per cent discoloration intensity (PDI) in all groundnut cultivars. *Aspergillus flavus* showed highest PDI in cultivars *viz*., GG 8 (77.75%), GG 2 (77.25%) and GG 11 (75%). *Aspergillus terreus* showed significantly lowest discoloration intensity in cultivar GG 20 (26.25%) which was at par with GG 5 (26.50%) and followed by GG 3 (29%) and GAUG 10 (29%).

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