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Establishing seed standard for seed health test in groundnut (*Arachis hypogea* L.) for *Aspergillus flavus*

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The relationship of *Aspergillus flavus* infection on seed quality was studied by artificial inoculation of *A. flavus* on seeds of groundnut cultivar VRI 2 collected from three major groundnut growing areas of TamilNadu viz., Vridhachalam, Thindivanam, Villupuram. *A. flavus* spores isolated from groundnut kernels were inoculated with 0, 0.25 and 0.5% infection on seed coat of disease free groundnut kernels after initial physiological and seed health evaluation and then stored in cloth bag at ambient condition and evaluated bimonthly. The seeds inoculated with 0.5% infection lost its viability (69%) at the end of storage period. Seeds with 0% infection maintained its viability at the end of storage period (72%). The seeds with 0.25% infection maintained germination up to 71% at the end of storage period. Storing the seeds with 0% infection is not at all possible, thus, the seeds with 0.25% of *A. flavus* infection could be the tolerable limit for safer storage of groundnut seeds.

Key words: *Aspergillus flavus*, groundnut cultivar, germination, storage period.

INTRODUCTION

Groundnut (*Arachis hypogea* L.) is an annual legume which is also known as peanut, earthnut, monkey-nut and goobers. It is the thirteenth most important food crop and fourth most important oilseed crop of the world (Reddy et al., 2011). India is one of the largest producers of oilseeds in the world and this sector occupies an important position in the agricultural economy. It is grown in more than 100 countries in the world. India, China, Nigeria, USA and Indonesia alone contribute 74% of the total world production. India contributes 19% of world production. It occupies an area of 6.41 million ha with a production of 9.824 million tonnes and possesses an average yield of 1.6 tonnes (Mehrotra, 2011).

Groundnut seeds are the nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used for culinary purpose. It is also used as an animal feed in the form of oil pressings, seeds, green material and straw and industrial raw material as oil cakes and fertilizer (Reddy et al., 2011). These multiple uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries.

About 90% of the crops all over the world are produced by using seeds. Quality seeds play a very important role for the production of healthy crop. It is reported that 25% of the world's crops are affected by mould or fungal growth. In India around 82% of groundnut produced is used for edible oil production, 12% as seed and 5% as feed (Mehrotra, 2011). The seeds are found to be responsible for disease transmission because they carry a number of pathogens, which get associated either in the field or in the post harvest storage condition (Manimurugan, 2003).

Fungi like *Aspergillus niger, Aspergillus flavus, Alternaria dianthicola, Curvularia lunata, Curvularia*

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pellescens, 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). Janardhan et al. (2011) found that *Aspergillus* is a common mould in tropical and sub tropical countries and causes aflatoxin contamination as a result of moulding of badly stored commodities, such as groundnut, cereal and cotton seeds. Groundnut being an oil seed boosts the vigour of pathogenic fungi resulting in bio-deterioration by the production of lipase. The tropical climate with high temperature and high relative humidity along with unscientific storage conditions adversely affect the preservation of cereal grains, oilseeds, etc., which lead to the total loss of seed quality.

Intensive crop improvement programme has resulted in the development of large number of high yielding varieties in different crops and more so in groundnut. Many of the varieties are in seed production chain in the organized sector. Thus, production and distribution of quality seeds to the farmers become increasingly important.

In a seed production programme, storage of seeds till the distribution during next season assumes paramount importance. Being an oil seed, groundnut losses its viability soon. Though, the initial seed quality and storage environment are important to prolong the shelf-life of seeds, the invasion of fungal pathogen also play a major role in decreasing the viability of a seed lot in groundnut. Therefore, production of disease free seeds for sustainable agriculture must be the order of the day.

It was evident that, inoculum pressure can be directly correlated with the intensity of disease development (Rajput et al., 2005). Increased percentage of pathogen infection can reduce the germination and vigour of barley (Harper and Lynch, 1981). 0.1 and 0.5% infection of *Alternaria brassica* and *M. phaseolina* in black gram and sesame, respectively decrease the germination percentage considerably (Anonymous, 2012b). In the Indian Minimum Seed Certification Standard, field and seed standards are prescribed for different crops. But seed standard for seed health status is prescribed for only a few crops. Moreover, in the forthcoming Seed Bill (2004), seed health is becoming a mandatory assessment for all seed samples. Since groundnut seeds are susceptible to fungal infection and losses its viability soon during storage, preliminary information on seed health status to fix a seed standard assumes greater importance. With these views, the present study was formed to identify the tolerance limit of *Aspergillus* infection which will not affect the germination below IMSCS.

MATERIALS AND METHODS

The seed samples of groundnut cv. VRI 2 collected from three major groundnut growing areas of Tamil Nadu viz, Vridhachalam, Tindivanam and Villupuram were used as base material for this study. The collected samples were hand sorted, cleaned thoroughly and tested for their initial quality including seed health status.

Germination

The germination test was conducted with 50 kernels in four replications in sand medium. The test conditions of 25±2ºC and 95±3% RH were maintained in a germination room. At the end of tenth day, the number of normal seedlings was counted and the mean was expressed as percentage. Seedling length was measured and expressed in cm (ISTA, 2010).

Vigour index

Vigour index was computed by adopting the method suggested by Abdul-Baki and Anderson (1973) and expressed as whole number.

Vigour index = Germination percentage × Seedling length in cm

Pathogen infection

Seed health testing for fungal infection was carried out using blotter technique for each sample. Ten kernels in ten replicates were placed equidistantly on three layered sterile blotter paper moistened with 0.2% 2,4-D solution in sterile Petri plates under aseptic condition and incubated at 20±2°C for 7 days with alternate cycles of 12 h in near ultraviolet light (NUV) range and for the remaining 12 h in dark. On the eighth day, the seeds were examined for the presence of fungal infection. The number of infected seeds was counted and the mean value was expressed in percentage (ISTA, 2010).

Preparation of solid medium of *Aspergillus flavus*

The fungi on seeds were isolated by transferring a single spore of *A. flavus* into potato dextrose agar medium in Petri plates with the help of stereo microscope (wild MPS 45) under aseptic condition and purified. The identification of these pathogens was further confirmed by preparing slides and examining them under compound microscope.

Preparation of spore suspension of *A. flavus* and artificial inoculation

Potato dextrose broth was used for the preparation of spores and mycelia of *A. flavus.* One hundred c.c. of potato broth was inoculated with fungus and incubated at 30°C in the dark for 10 days under stationary condition. The fungal biomass was transferred to a waring blender containing distilled water and blended for 30 s at a speed of 2000 rpm. The suspension containing spores and mycelia were inoculated on shelled, hand sorted, cleaned kernels by smearing uniformly on seed coat (Amrit and Singh, 1998). This was considered as 100% of *A. flavus* infected seed sample.

Preparation of various percentages of *A. flavus* infected seed sample

From the 100% infected sample, various percentages (0.25, 0.5, 0.75, 1, 2, 3, 4 and 5%) of infected seed samples were prepared by mixing the good, healthy seeds with infected seeds. They were

Table 1. Concentrations of *A. flavus* infection on seed quality parameters.

Pathogen infection (%)	Germination (%)	Shoot length (cm)	Root length (cm)	Dry matter production (g)	Vigour index
Control (0)	85 (67.21)	14.5	15.8	3.46	2576
0.25	82 (64.89)	14.1	15.5	3.44	2427
0.50	78 (62.02)	13.5	15.1	3.35	2231
0.75	75 (60.00)	13.5	14.6	3.42	2108
1.00	71 (57.41)	12.7	13.9	3.26	1889
2	68 (55.55)	12.4	13.4	3.21	1754
3	64 (53.13)	12.1	13.1	3.18	1613
4	62 (51.94)	11.9	12.8	2.88	1531
5	60 (50.76)	11.7	12.5	2.52	1452
SEd	0.991	0.154	0.170	0.015	33.81
CD (P = 0.5)	2.549**	0.349**	0.384**	0.034**	76.501**

Figures in parentheses indicate arc sine values.

named as follows:

T_1 - Control (Uninoculated or 0 % infection),
T_2 - 0.25% infected seed sample,
T_3 - 0.5% infected seed sample,
T_4 - 0.75% infected seed sample,
T_5 - 1% infected seed sample,
T_6 - 2% infected seed sample,
T_7 - 3% infected seed sample,
T_8 - 4% infected seed sample,
T_9 - 5% infected seed sample.

Evaluation of performance of artificially inoculated seeds

The artificially inoculated kernels of various percentages of infection were evaluated for the seed quality as detailed as follows:

Fixing inoculum threshold level

The inoculum level which will maintain minimum germination percentage of 70 (as given in IMSCS) at the end of the storage period will be fixed as the tolerance level of infection for groundnut seeds.

Statistical analysis

The data pertaining to the observations recorded in the laboratory were analyzed using completely randomized design adopting the procedure as described by Panse and Sukhatme (1967). Whenever necessary, the suitable transformations were made before analysis. The critical difference (CD) was computed at 5% probability. Significance and non-significance were denoted as * and NS, respectively.

RESULTS

The experimental results of storage studies and fixing inoculum threshold level are detailed hereunder.

Concentrations of *Aspergillus flavus* infection on seed quality parameters

Different concentrations of *A. flavus* infection showed significant differences for germination, shoot length, root length, dry matter production and vigour index (Table 1). With increased concentrations of 2 to 5%, the above quality parameters recorded lower value, whereas, the lower level of infection of 0.25 to 1.0% recorded the higher values of seedling quality parameters. Among different concentrations, 0.25% recorded higher germination of 82%, whereas 5.0% inoculated seeds recorded 60% germination and vigour index 2427 and 1452. The seeds inoculated with 0.25 and 0.50% were taken for storage studies to fix tolerance level of *A. flavus* based on germination and vigour index.

Performance of artificially inoculated seeds during storage

Seed quality characteristics

Germination (%): Significant differences were observed for germination among locations, concentrations, period of storage and their interactions. Seeds collected from Tindivanam (L_2) recorded maximum germination (78%) followed by the seeds collected from Villupuram (L_1) (75%) and Vridhachalam (L_3) (70%) (Table 2). Among concentrations, seeds with 0.5% infection (C_3) recorded the minimum germination (75%) and seeds with 0.25% infection (C_2) recorded the maximum (76%). Seeds treated with Carbendazim (T_2) showed higher germination (77%) when compared to non-treated control (T_1) which showed 76%. Germination percent reduced from first month (P_0) to the end of storage period (P_6) and ranged from 83 to 70%. Irrespective of locations, non-inoculated seeds treated with carbendazim recorded 72%

Table 2. Germination (%) of artificially inoculated seeds of groundnut cv. VRI 2 collected from different locations during storage.

L/C/T	Period of storage (months)						SEd	CD (P = 0.05)
	P_0	P_2	P_4	P_6	Mean			
$L_1C_1T_1$	85(67.21)	78(62.02)	73(58.69)	70(56.79)	77(61.00)	L	0.290	0.579**
$L1C_1T_2$	85(67.21)	81(64.15)	75(60.00)	71(57.41)	78(62.02)			
Mean	85(67.21)	80(63.08)	74(59.34)	71(57.10)	77(61.54)	C	0.289	0.577**
$L_1C_2T_1$	82(64.89)	76(60.66)	72(58.05)	69(56.16)	75(59.86)			
$L_1C_2T_2$	83(65.65)	77(61.34)	73(58.69)	70(56.79)	76(60.53)	T	0.237	0.669**
Mean	83(65.27)	77(61.00)	73(58.37)	70(56.47)	75(60.20)			
$L_1C_3T_1$	78(62.02)	74(59.34)	71(57.41)	68(55.55)	73(58.56)	P	0.335	1.003**
$L_1C_3T_2$	79(62.72)	76(60.66)	72(58.05)	69(56.16)	74(59.34)			
Mean	79(62.37)	75(60.00)	72(57.73)	69(55.85)	73(58.95)	LC	0.503	0.819**
Mean	82(64.89)	77(61.34)	73(58.50)	70(56.47)	75(60.20)			
$L_2C_1T_1$	86(68.02)	82(64.89)	76(60.66)	72(58.05)	79(62.72)	LT	0.411	NS
$L_2C_1T_2$	86(68.02)	83(65.65)	78(62.02)	74(59.34)	80(63.65)			
Mean	86(68.02)	83(65.27)	77(61.34)	73(58.69)	80(63.15)	LP	0.581	1.159**
$L_2C_2T_1$	85(67.21)	79(62.72)	74(59.34)	71(57.41)	77(61.54)			
$L_2C_2T_2$	85(67.21)	80(63.43)	76(60.66)	72(58.05)	78(62.23)	CT	0.413	NS
Mean	85(67.21)	80(63.08)	75(60.00)	72(57.73)	78(61.89)			
$L_2C_3T_1$	83(65.65)	77(61.34)	73(58.69)	69(56.16)	76(60.33)	CP	0.578	1.157**
$L_2C_3T_2$	82(64.89)	78(62.02)	75(60.00)	70(56.79)	76(60.86)			
Mean	83(65.27)	78(61.68)	74(59.34)	70(56.47)	76(60.60)	TP	0.474	0.649**
Mean	85(66.81)	80(63.29)	75(60.20)	71(57.60)	78(61.89)			
$L_3C_1T_1$	82(64.89)	79(62.72)	74(59.34)	71(57.60)	77(61.00)	LCT	0.712	NS
$L_3C_1T_2$	82(64.89)	80(63.43)	75(60.00)	72(58.05)	77(61.00)			
Mean	82(64.89)	80(63.08)	75(59.67)	72(57.73)	77(61.27)	LCP	0.007	1.378**
$L_3C_2T_1$	81(64.15)	77(61.34)	73(58.59)	70(56.79)	75(60.20)			
$L_3C_2T_2$	82(64.89)	78(62.02)	74(59.34)	71(57.41)	76(60.89)	LTP	0.822	NS
Mean	82(64.52)	78(61.68)	74(59.01)	71(57.10)	76(60.53)			
$L_3C_3T_1$	80(63.43)	76(60.66)	72(58.05)	68(55.55)	74(59.34)	CTP	0.825	NS
$L_3C_3T_2$	80(63.43)	77(61.34)	73(58.69)	69(56.16)	75(59.86)			
Mean	80(63.43)	77(61.00)	73(58.37)	69(55.85)	74(59.60)			
Mean	81(64.30)	78(61.89)	74(59.01)	70(56.91)	76(60.46)	LCTP	1.424	NS
Mean	83(65.34)	78(62.16)	74(59.21)	70(56.97)				

Table 2. Contd.

Mean values											
L_1	L_2	L_3	C_1	C_2	C_3	T_1	T_2	P_0	P_2	P_4	P_6
75	78	70	78	76	75	76	77	83	78	74	70

Interaction table				
	P_0	P_2	P_4	P_6
C_1T_1	84	80	74	70
C_1T_2	84	81	76	72
C_2T_1	83	77	73	70
C_2T_2	83	78	74	71
C_3T_1	80	76	72	68
C_3T_2	80	77	73	69

Figures in parentheses indicate arc sin value. L_1 – Vridhachalam, L_2 – Tindivanam, L_3 – Villupuram, C_1 – Control (Non-inoculated), C_2 – 0.25% inoculated, C_3 – 0. 5% inoculated, T_1 – Control (Non-treated), T_2 – Carbendazim treated.

germination and non-treated seeds recorded 70% germination at the end of storage period, whereas 0.5% infected seeds recorded 69 and 68% germination under treated and non-treated conditions. The seeds inoculated with 0.25% inoculum recorded higher germination of 71 and 70% germination under treated and non-treated condition at the sixth month of storage.

Vigour index: Vigour index was highly significant among locations, concentrations, treatments and their interactions. L_2 recorded the higher vigour index (2420) and L_3 recorded the lower (2081) (Table 3). Among concentrations, C_1 showed higher vigour (2285) than C_3 (2115). Among treatments, T_2 showed more vigour index value (2225) than T_1 (2178). Irrespective of locations, non-inoculated seeds recorded the vigour index of 2036 and 1964 under treated and non-treated conditions, respectively at sixth month of storage. Whereas, 0.25% inoculated seeds showed 1921 and 1963 vigour index at the end of storage period under non-treated and treated conditions, respectively followed by 0.5% inoculated seeds (1848 and 1888).

***Aspergillus flavus* infection (%):** Percentage of *A. flavus* infection was highly significant among locations, concentrations, treatments, period of storage and their interactions (Table 4). L_1 recorded maximum percentage of infection (8.3%), whereas L_2 showed the minimum (6.7%). Different concentrations of artificial inoculations significantly influenced the infection during storage. C_3 recorded the maximum infection (10.1%) whereas, C_2 recorded the minimum (6.9%) followed by C_1 (5.8%). Carbendazim treated seeds showed less infection (6.9%) than non-treated control (9.8%). Irrespective of all factors, infection increased from 0.25 to 16.7% as storage period increased. Seeds stored with 0.5% infection recorded 18.3 and 23.3% infection at the end of storage period under treated and non-treated condition, respectively. Whereas, non-inoculated seeds recorded 8.3 and 18.3% infection at the end of storage period under treated and non-treated condition, respectively followed by 0.25% inoculated seeds (11.7 and 20%).

DISCISSION

Different concentrations of *A. flavus* infection showed significant differences for germination and vigour index. With increased concentrations of 2 to 5%, the above quality parameters recorded lower value, whereas, the lower level of infection of 0.25 to 1.0% recorded the higher values (Figure 1). Among different concentrations, 0.25% recorded higher germination of 82%, whereas 5.0% inoculated seeds recorded 60% germination and vigour index 2427 and 1452. The seeds inoculated with 0.25 and 0.50% were taken for storage studies to fix tolerance level of *A. flavus* based on germination and vigour index. Inoculum threshold, become a very important factor in seed pathology and plant disease control by the use of clean seed. Inoculum threshold of seed borne pathogens is the amount of seed infection or infestation with plant pathogens that will cause a disease in the field under a conducive environment and lead to economic losses. It is important to establish the inoculum threshold level, when clean seed is used as a disease control measure in quality seed production.

The healthy seed is an effective prophylactic measure to ensure the effective control of spread of seed borne pathogen and production of disease free crop in the field. The field standards for disease free seed production have been given in IMSCS. But the seed standards for seed health have been given for very few crops in India (Tunwar and Singh, 1988). Groundnut being an important oilseed is much affected by storage fungi and fixing up of

Table 3. Vigour index of artificially inoculated seeds of groundnut cv. VRI 2 collected from different locations during storage.

Month/Lots	Concentrations	Treatments	P_0	P_2	P_4	P_6	Mean		SEd	CD (P = 0.05)
L_1		T_1	2550	2239	2037	1849	2168	L	29.2	57.7**
	C_1	T_2	2550	2365	2108	1890	2228			
		Mean	2550	2302	2072	1869	2198	C	29.5	57.9**
		T_1	2427	2151	1944	1787	2077			
	C_2	T_2	2465	2210	2022	1834	2132	T	23.9	47.3**
		Mean	2446	2180	1983	1810	2105			
		T_1	2239	2065	1889	1734	1981	P	33.7	NS
	C_3	T_2	2267	2113	1922	1773	2018			
		Mean	2253	2089	1905	1753	2000	LC	50.5	100.3**
	Mean		2416	2190	1987	1811	2101			
L_2		T_1	2881	2608	2326	2146	2490	LT	41.3	81.9**
	C_1	T_2	2881	2689	2434	2227	2557			
		Mean	2881	2648	2380	2186	2524	LP	58.6	116.2**
		T_1	2805	2473	2227	2095	2400			
	C_2	T_2	2788	2552	2318	2131	2447	CT	41.5	82.1**
		Mean	2796	2512	2272	2113	2423			
		T_1	2656	2364	2168	1994	2295	CP	58.3	115.9**
	C_3	T_2	2624	2426	2250	2037	2334			
		Mean	2640	2395	2209	2015	2314	TP	47.6	94.6**
	Mean		2772	2518	2287	2105	2420			
L_3		T_1	2312	2204	2028	1897	2110	LCT	71.4	141.9**
	C_1	T_2	2312	2224	2078	1993	2151			
		Mean	2312	2214	2053	1945	2131	LCP	100.9	200.7**
		T_1	2268	2118	1978	1883	2061			
	C_2	T_2	2312	2161	2028	1924	2106	LTP	82.4	163.9**
		Mean	2290	2139	2003	1903	2084			
		T_1	2216	2075	1944	1816	2012	CTP	82.4	163.9**
	C_3	T_2	2240	2118	1978	1856	2048			
		Mean	2228	2096	1961	1836	2030			
	Mean		2276	2150	2005	1894	2081	LCTP	142.6	283.9**
Mean			2488	2286	2093	1937				

Mean values

L_1	L_2	L_3	C_1	C_2	C_3	T_1	T_2	P_0	P_2	P_4	P_6
2101	2420	2081	2285	2204	2115	2178	2225	2489	2286	2093	1937

Interaction table

	P_0	P_2	P_4	P_6
C_1T_1	2581	2350	2130	1964
C_1T_2	2581	2426	2206	2036
C_2T_1	2500	2247	2049	1921
C_2T_2	2521	2307	2122	1963
C_3T_1	2370	2168	2000	1848
C_3T_2	2377	2219	2050	1888

L_1 – Vridhachalam, L_2 – Tindivanam, L_3 – Villupuram, C_1 – Control (Non-inoculated), C_2 – 0.25% inoculated, C_3 – 0. 5% inoculated, T_1 – Control (Non-treated), T_2 – Carbendazim treated.

Table 4. *A. flavus* infection (%) on artificially inoculated seeds of groundnut cv. VRI 2 collected from different locations during storage.

Periods of storage			P_0	P_2	P_4	P_6	Mean		SEd	CD (P = 0.05)
Lots	**Concentrations**	**Treatments**								
L_1		T_1	0 (0.00)	5(0.77)	10 (1.04)	20 (1.32)	8.8 (0.99)	L	0.367	0.726**
	C_1	T_2	0 (0.00)	0 (0.00)	5 (0.77)	10 (1.04)	3.8 (0.68)			
		Mean	0 (0.00)	2.5 (0.54)	7.5 (0.92)	15 (1.20)	6.3 (0.86)	C	0.365	0.723**
		T_1	0.25 (0.11)	5 (0.77)	15 (1.20)	20 (1.32)	10.1 (1.04)			
	C_2	T_2	0.25 (0.11)	2 (0.47)	5 (0.77)	10 (1.04)	4.3 (0.72)	T	0.300	0.593**
		Mean	0.25 (0.11)	3.5 (0.65)	10 (1.04)	15 (1.20)	7.2 (0.91)			
		T_1	0.5 (0.17)	10 (1.04)	20 (1.32)	25 (1.41)	13.9 (1.17)	P	0.424	0.839**
	C_3	T_2	0.5 (0.17)	5 (0.77)	10 (1.04)	20 (1.32)	8.9 (0.99)			
		Mean	0.5 (0.17)	7.5(0.92)	15 (1.20)	22.5(1.37)	11.4 (1.09)	LC	0.636	1.259**
	Mean		0.25 (0.11)	4.5(0.74)	10.8 (1.07)	17.5 (1.26)	8.3 (0.96)			
L_2		T_1	0 (0.00)	5 (0.77)	10 (1.04)	15 (1.20)	7.5 (0.92)	LT	0.519	1.027**
	C_1	T_2	0 (0.00)	0 (0.00)	5 (0.77)	5 (0.77)	2.5 (0.54)			
		Mean	0 (0.00)	2.5(0.54)	7.5 (0.92)	10 (1.04)	5.0 (0.77)	LP	0.735	1.453**
		T_1	0.25 (0.11)	5 (0.77)	10 (1.04)	20 (1.32)	8.8 (0.99)			
	C_2	T_2	0.25 (0.11)	0 (0.00)	5 (0.77)	10(1.04)	3.8(0.68)	CT	0.517	1.024**
		Mean	0.25 (0.11)	2.5(0.54)	7.5 (0.92)	15(1.20)	6.3(0.86)			
		T_1	0.5 (0.17)	5 (0.77)	15 (1.20)	20(1.32)	10.1 (1.04)	CP	0.735	1.453**
	C_3	T_2	0.5 (0.17)	5 (0.77)	10 (1.04)	15(1.20)	7.6(0.93)			
		Mean	0.5 (0.17)	5 (0.77)	12.5(1.13)	17.5(1.26)	8.9(0.99)	TP	0.600	1.187**
	Mean		0.25 (0.11)	3.3 (0.63)	9.2 (1.00)	14.2 (1.18)	6.7 (0.88)			
L_3		T_1	0 (0.00)	5 (0.77)	10 (1.04)	20 (1.32)	8.8 (0.99)	LCT	0.900	1.780**
	C_1	T_2	0 (0.00)	0 (0.00)	5 (0.77)	10 (1.04)	3.8 (0.68)			
		Mean	0 (0.00)	2.5 (0.54)	7.5 (0.92)	15 (1.20)	6.3 (0.86)	LCP	1.273	2.518**
		T_1	0.25 (0.11)	5 (0.77)	10 (1.04)	20 (1.32)	8.8 (0.99)			
	C_2	T_2	0.25 (0.11)	3 (0.60)	5 (0.77)	15 (1.20)	5.8 (0.83)	LTP	1.039	NS
		Mean	0.25 (0.11)	4 (0.69)	7.5 (0.92)	17.5 (1.26)	7.3 (0.91)			
		T_1	0.5 (0.17)	5 (0.77)	15 (1.20)	25 (1.41)	11.4 (1.09)	CTP	1.041	2.055**
	C_3	T_2	0.5 (0.17)	5 (0.77)	10 (1.04)	20 (1.32)	8.9 (0.99)			
		Mean	0.5 (0.17)	5 (0.77)	12.5 (1.13)	22.5 (1.37)	10.1 (1.04)			
	Mean		0.25 (0.11)	3.8 (0.68)	9.2 (1.00)	18.3 (1.28)	7.9 (0.94)	LCTP	1.801	NS
Mean			0.25 (0.11)	3.9 (0.69)	9.7 (1.02)	16.7 (1.24)				

Table 4. Contd.

Mean values											
L_1	L_2	L_3	C_1	C_2	C_3	T_1	T_2	P_0	P_2	P_4	P_6
8.3	6.7	7.9	5.8	6.9	10.1	9.8	6.9	0.25	3.9	9.7	16.7

Interaction table				
	P_0	P_2	P_4	P_6
C_1T_1	0.0	5.0	10.0	18.3
C_1T_2	0.0	0.0	5.0	8.3
C_2T_1	0.3	5.0	11.7	20.0
C_2T_2	0.3	2.0	5.0	11.7
C_3T_1	0.5	6.7	16.7	23.3
C_3T_2	0.5	5.0	10.0	18.3

L_1 – Vridhachalam, L_2 – Tindivanam, L_3 – Villupuram, C_1 – Control (Non-inoculated), C_2 – 0.25% inoculated, C_3 – 0. 5% inoculated, T_1 – Control (Non-treated), T_2 – Carbendazim treated.

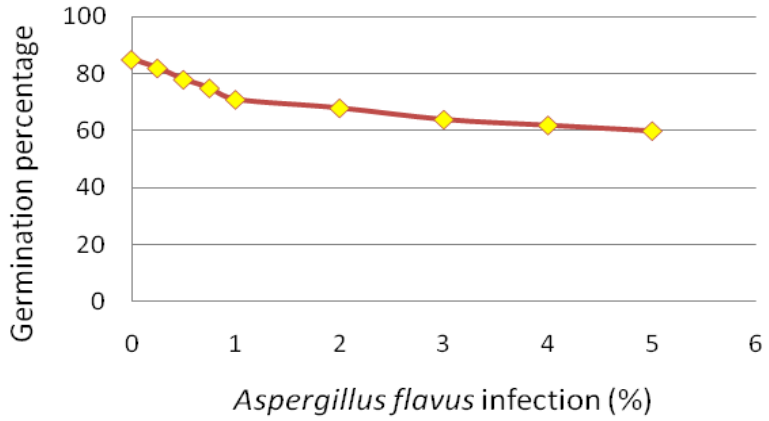

Figure 1. Different concentration of *A. flavus* infection on seed germination.

minimum tolerance level of pathogen infection become increasingly important (Bhale et al., 2001).

Seeds inoculated with 0.25% of *A. flavus* recorded germination of 70% at the end of storage period of 6 months from the initial germination of 83% (Figure 2). The vigour index (1921) was also maintained up to the end of storage period irrespective of location of seed production. The non-inoculated seeds also recorded 70% germination. Under carbendazim treated condition, the non-inoculated seeds and 0.25% inoculated

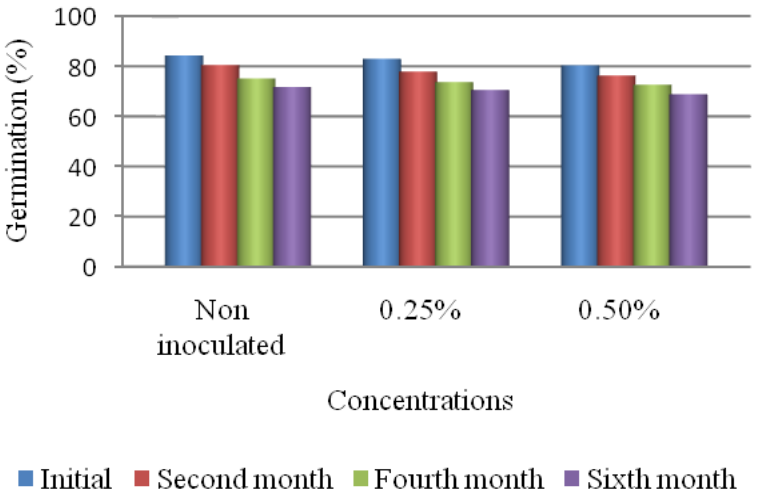

Figure 2. Influence of different concentrations of artificial inoculation of *A. flavus* on germination of groundnut cv. VRI 2 during storage.

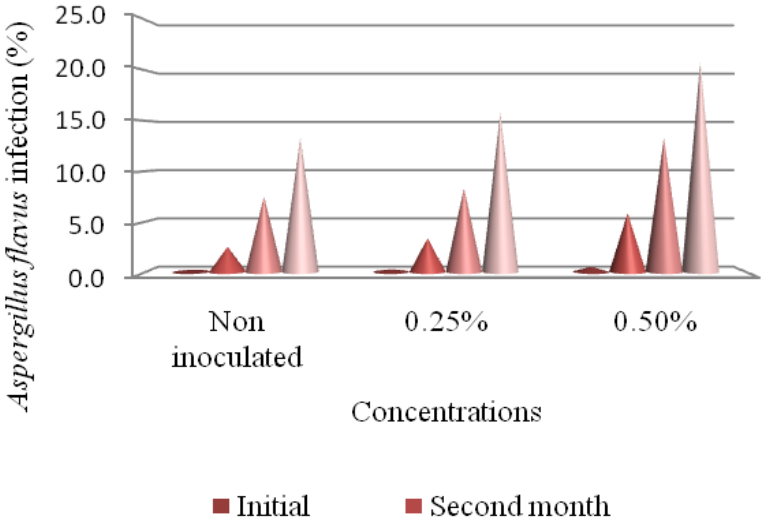

Figure 3. Change in the development of *A. flavus* in artificially inoculated seeds of groundnut cv. VRI2 during storage.

seeds recorded the germination of 72 and 71%, respectively at the end of storage period irrespective of location of sed production. 0.25% inoculated seeds recorded 9% reduction in germination, whereas the non-inoculated seeds recorded only 7% reduction. Similar results were obtained by Harper and Lynch (1981) in barley, Imolehin (1983) in paddy and Pant (2011) in coriander.

The species of *Aspergillus* are externally seed borne in nature, while the seeds stored as kernels the surface contamination may occur and it will enhance the fungal growth led to decrease in germinability and other seed quality characters (Charles, 1979). Externally seed borne pathogen may compete with the embryo for oxygen supply and thus inhibits the germination (Harper and Lynch, 1981).

The seeds with 0.25% inoculation recorded 20% of *A. flavus* infection at the end of storage period (Figure 3). Under treated condition, the fungal infection decreased to 11.72%. Similar results were obtained by Manimurugan (2003) in black gram. The increase in seed germination and vigour in the treated seeds may be due to the control of fungal mycelial growth (Mohanna and Sharma, 1991; Habib et al., 2007).

In Ethiopia, seed standard for different oilseeds crops were suggested as 0% infection for castor, mustard,

linseed, safflower, rape seed, sesame and sunflower (Anonymous, 2012a). In Bangladesh, Alternaria blight (*A. brassica*) infection, seedling blight (*M. phaseolina*) in black gram and sesame stem rot infection (*M. phaseolina*) can be acceptable up to 0.1 and 0.5% for foundation and certified seeds, respectively (Anonymous, 2012b).

The non-inoculated healthy seeds which recorded germination of 84% showed 4% reduction in germination after 2 months of storage, whereas 0.25% inoculated seeds which recorded a germination of 83% showed 6% reduction in germination with the development of 5.0% fungal infection after 2 months of storage. It was evident that inoculum pressure can be directly correlated with the intensity of disease development as reported by Rajput et al. (2005). Under treated condition, the percentage of reduction in germination was minimized to 3 and 5% in non-inoculated and 0.25% inoculated seeds, respectively. In UK, the acceptable level of *Ascochyta* spp. infection in green pea was 0 and 10% and in dry pea seeds it was 7 and 15% under non-treated and treated conditions, respectively (Anonymous, 1985).

The results of the present investigation showed that the seeds inoculated with 0.25% of *A. flavus* and treated with carbendazim maintained 71% of germination at the end of storage period which is just above the minimum seed standard as recommended by IMSCS. Thus, 0.25% infection level of *A. flavus* may be considered as permissible tolerance level for groundnut seed.

REFERENCES

Abdul-Baki AA, Anderson JD (1973). Vigour determination in soybean seed by multiple criteria. Crop Sci. 13:630-633.

Amrit S, Singh RD (1998). Role of seed borne inoculum in Development of Charcoal rot of cow pea. J. Mycol. Pl. Pathol. 28(2):193-195.

Anonymous (1985). The vegetable seed regulations. 1985 no 976. Her Majesty's stationery office, London.

Anonymous (2012a). WANA Catalogue of Field and Seed Standards.

Anonymous (2012b). BADC: Bangladesh Agricultural Development Corporation.

Bhale MS, Khare D, Raut ND, Singh D (2001). Seed borne diseases objectionable in seed production and their management, Scientific publishers (India), Jodhpur. P. 21.

Charles CB (1979). The effects of the shell on the viability and moisture content of peanut seeds in storage under alternating relative humidities. Seed Res. 7(1):1-4.

Chavan AM, Kakde RB (2008). Studies on abnormal oilseeds mycoflora from Marathwada region. Bionano Frontier 2(2):101-104.

Habib A, Sahi ST, Ghazanfar MU, Ali S (2007). Location of seed-borne mycoflora of eggplant (*Solanum melongena* L.) in different seed components and impact on seed germinability. Int. J. Agric. Biol. 9:514-516.

Harper SHT, Lynch JM (1981). Effects of fungi on barley seed germination. J. Gen. Microbiol. 122:55-60.

Imolehin ED (1983). Rice seed-borne fungi and their effect on seed germination. Plant Dis. 67:1334-1336.

International Seed Testing Association (2010). International Rules for Seed Testing: edition 2010. ISTA, Bassersdorf, Switzerland.

Janardhan AD, Subramanyam A, Praveen K, Reddi Pradeep M, Narasimha G (2011). Aflatoxin impacts on germinating seeds. Ann. Biol. Res. 2(2):180-188.

Manimurugan C (2003). Pathogen free seed production in black gram (*Vigna mungo* (L.) Hepper). M.Sc. (Ag.) Thesis, TamilNadu Agricultural University, Coimbatore. Tamil Nadu.

Mehrotra N (2011). Commodity Specific Study in groundnut. Department of Economic Analysis and Research, National Bank for Agriculture and Rural Development, Mumbai. pp. 7-8.

Mohanna C, Sharma JK (1991). Seed pathology of forest tree species in India - present status practical problems and future prospects. Commonw. For. Rev. 70(3):133-151.

Panse VG, Sukhatme PV (1967). Statistical methods for Agricultural workers. ICAR., Pub., New Delhi.

Pant R (2011). Seed Mycoflora of Coriander and Effect of Some Fungal Metabolite on Seed Germination and Seedling Growth. Asian J. Exp. Biol. Sci. 2(1):127-130. Anonymous (2012). BADC: Bangladesh Agricultural Development Corporation.

Rajput AM, Mumtaz A, Pathan A, Mubeen I, Sarwar SG, Khalil A, Khanzada (2005). Studies on seed-borne fungi of wheat in sindh province and their effect on seed germination. Pak. J. Bot. 37(1):181-185.

Reddy SEC, Sudhakar C, Eswara Reddy NP (2011). Aflatoxin contamination in groundnut induced by *aspergillus flavus* type fungi: a critical review. Int. J. Appl. Biol. Pharm. Technol. 2(2):180-192.

Tunwar NS, Singh SV (1988). Seed standard for foundation and certified seeds based on maximum percent seed infection, handbook of seed testing. pp. 128-129.