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Seed Health Evaluation of Pea Varieties by Incubation Methods

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Like other crops, pea varieties were also harbour the seed borne mycoflora resulted in less germination and plant vigour index. Six varieties of pea and one local variety were taken in the present investigation to assess the seed health by incubation methods. It was found that varying degree and type of mycoflora associated with pea seeds were recorded. The mycoflora recorded were *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Trichoderma* sp., *Basidiophora* sp., *Chaetomium* sp., *Aspergillus fumigatus*, *Alternaria* sp., *Curvularia* sp., *Fusarium* sp., *Rhizopus* sp. and *Rhizoctonia* sp. In all these methods, poor germination was observed in local variety, may be due to presence of seed borne mycoflora with higher frequencies whereas Shubhra variety has higher germination due to lower frequencies of detected mycoflora as compared to other varieties of pea taken in the study.

Introduction

Pea is the third most important pulse crop at global level, after dry bean and chickpea and third most popular *Rabi* pulse of India after chickpea and lentil. It is originated from Mediterranean Region of Southern Europe and Western Asia. It provides a variety of vegetarian diet hence liked throughout the world. The mature seeds are used as whole or split into dal and put to use in various ways for human consumption. Beside vegetable purposes, it is also grown as a forage crop for cattle and cover crop to prevent soil erosion but mainly for matured seed for human consumption. The seeds are highly nutritious as it contains about 22.5% protein, 64 mg/100g calcium, 1.8% fat, 4.8 mg/100g iron, 62.1% carbohydrate and 11% moisture.

Healthy seed is the foundation of healthy plant, a necessary condition for good yield (Diaz *et al.*, 1998). Seed health is affected by various factors, among which the most important is seed borne fungi that cause reduction in seed germination and seed vigour. Germination, growth and productivity of crop plants are affected by the infection of various mycoflora. 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 the development of disease at later stages of plant growth of systemic or local infection.

Ten different fungi namely *Alternaria* sp., *Aspergillus flavus*, *A. niger*, *Chaetomium*

globosum, *Fusarium oxysporum*, *Fusarium* sp., *Cladosporium cladosporioides*, *Rhizoctonia solani*, *Penicillium* sp. and *Curvularia lunata* were associated with pea seeds (Khan *et al.*, 2006).

Till date, seed health evaluation aspects like mycoflora associated, their effect on seedling vigour index, embryo infection, transmission and management not studied well and documented of different pea varieties of Chhattisgarh. Therefore, an attempt is taken to carry out the present investigation to assess the seed health of pea varieties by incubation methods.

Materials and Methods

Unless and otherwise mentioned for each experiment, 400 seeds were used. In general, the petridish with seeds were incubated at $22\pm 1^{\circ}\text{C}$ under a 12 hours' dark and light cycle with NUV light for seven days. Observations were recorded seven days after seeding for the type of mycoflora associated and its frequency. The organisms were observed under the stereobinocular microscope on seeds for their habit characters and confirmed under compound microscope.

The associated mycoflora were identified with the help of standard literature like Illustrated Genera of Imperfect Fungi (H.L. Barnett, 1962), More Dematiaceous Hypomycetes (M.B. Ellis, 1976) and A Pictorial Guide to the Identification of Seed borne Fungi of Sorghum, Pearl Millet, Finger Millet, Chickpea, Pigeonpea and Groundnut (ICRISAT, 1978).

Several methods of seed health testing have been developed for the detection of fungi associated with seeds. Some of the most suitable procedures have been recommended by the International Seed Testing Association (ISTA) (Anon., 1966). The methods to detect

microbes in or on the seed vary quite markedly depending on the location of the microbes and the mode of seed transmission (Neergaard, 1977) and the particular group to which the microbe belongs.

The following standard methods recommended were used in the present study for seed health evaluation of different varieties of pea.

Standard blotter method (ISTA, 1976)

Agar plate method (Muskett and Malone, 1941)

Roll paper towel method (Yaklich, 1985)

Deep freeze method (Limonard, 1968)

Standard blotter method

This method was used to detect the presence of fungi on or in the seeds after incubation. By this method, the fast growing fungi were better detected than the slow growing ones. In each inter-fitting plastic petri plate, two good quality blotter papers of the same diameter were kept and moistened with sterilized distilled water.

In each plate, ten seeds were placed on the moistened blotters in such a manner that nine seeds formed the outer circle and one at the center. For each lot of seed, 40 replicated plates were maintained (total of 400 seeds tested for each lot of seed). Incubate the plate at $22\pm 1^{\circ}\text{C}$ for seven days in alternating cycles of 12 hours' darkness and 12 hours' light in NUV.

Observations were recorded as described earlier. All seeds of the outer ring were examined first, finally the seed in the centre of the dish and expressed in per cent seed mycoflora associated, individually.

The frequency of the fungus was calculated by the following formula

$$\frac{\text{No. of seeds containing a particular fungus}}{\text{Total seeds examined}} \times 100$$

$$\text{Relative abundance} = \frac{\text{Total no. of colonies of a fungus on seeds}}{\text{Total no. of colonies of all fungus}} \times 100$$

Agar plate method

Potato dextrose agar medium (15-20 ml) was poured in each sterilized petri plate. To avoid bacterial contamination, a little amount of Streptomycin sulphate was added in the medium at the time of pouring. Seeds of each lot were surface sterilized with 1.0% NaOCl solution for 30 seconds and immediately washed twice with sterile distilled water thoroughly to remove NaOCl solution that adhered if any. Seeds were placed on the previously poured PDA medium in Petri plate in such a way that nine seeds in the outer circle and one at the centre. For each lot, 40 replicated plates were maintained and incubated at 22±1°C under alternate cycles of 12 hrs dark and 12 hrs light in NUV. Observations were recorded as described earlier.

Roll paper towel method

The seed were placed on moist paper towel (50-100) at equidistance and covered with another moist paper towel and rolled carefully without disturbing the already arranged seeds. Tie the towel with rubber band at both the ends. To avoid water loss, use wax coated paper or polythene rapping the rolled towels containing seeds. Incubate it for four to five days at room temperature. Examine normal and abnormal seedlings, ungerminated seeds, cause of abnormalities and failure in germination by naked eye and presence of

mycoflora by stereo-binocular microscope. The observations were recorded for-

Normal seedlings

Show the potential for continued development into satisfactory plants when grown in good quality soil and under favorable conditions of moisture, temperature and light.

Categories

Intact seedlings

Seedlings with all their essential structures well developed, complete in proportion and healthy.

Seedlings with slight defects

Seedlings showing slight defects of their essential structures provided they show a satisfactory and balanced development comparable to that of intact seedlings of the same test.

Seedlings with secondary infection

Seedlings as described above but have been affected by fungi or bacteria from sources other than the parent seed.

Abnormal seedlings

Do not show the potential to develop into a normal plant when grown in good quality soil and under favorable condition of moisture, temperature and light.

Categories

Damaged

Seedlings with any of the essential structures missing or badly and irreparably damaged that balance development cannot be expected.

Deformed or unbalanced

Seedlings with weak development or physiological disturbances or in which essential structures are deformed or out in proportion.

Decayed

Seedlings with any of their essential structures so diseased or decayed

As a result of primary infection that normal development is prevented.

Ungerminated seeds

Did not germinate at the end of the test period.

Categories

Hard seeds

Seeds which have not absorbed water thus remain. Hard after the end of the test period.

Fresh seeds

Seeds able to imbibe water but which failed to germinate under the condition of the germination test remain clean and firm and have the potential to develop into a normal seedling.

Dead seeds

Seeds at the end of the test period are neither hard nor fresh,

Failed to proof a seedling; usually soft, discolored, frequently moldy.

Others

Empty, embryo less seeds, insect damaged seeds.

Deep freeze method

It was a modification of standard blotter method. The seeds were plated on blotters moistened with a solution containing 0.2% Streptopenicillin (to avoid bacterial contamination) and incubated for 24 hours under normal conditions in growth chamber. Plates were further incubated at $10 \pm 1^{\circ}\text{C}$ for three days and then transferred to deep freezer (-20°C) under complete darkness for 24 hours. Plated were again incubated at $20-25^{\circ}\text{C}$ for 5-7 days. Observations were recorded as described earlier.

Results and Discussion

Standard blotter method

Seed lots of different pea varieties were evaluated for the associated seed borne mycoflora by using standard blotter method and data presented in table 1 indicates that maximum frequency of mycoflora were recorded from the seed lot of local variety (111.25%) with lowest germination percentage (75.76) and six mycoflora were detected as *Aspergillus flavus* (21%), *Aspergillus niger* (10.50%), *Aspergillus fumigatus* (6%), *Alternaria* sp. (33.50%), *Chaetomium* sp.(24.75%) and *Rhizopus* sp. (15.5%). This was followed by KPMR 400 seed lot (97.50%) and five mycoflora were detected as *Aspergillus flavus* (29.25%), *Aspergillus niger* (9.25%), *Trichoderma* sp. (15.00%), *Alternaria* sp. (39.25%) and *Chaetomium* sp. (4.75%) with germination percentage 83.50.

In the seed lot of Ambika, Indira matar, IPFD 10-12 and Paras frequency of mycoflora associated were 95.50%, 87.75%, 80.75% and 77%, respectively. In Shubhra variety minimum frequency of mycoflora (65.5%) were recorded and mycoflora detected as *Aspergillus flavus* (15.5%), *Aspergillus niger*

(18.5%), *Aspergillus fumigatus* (4.5%) and *Chaetomium* sp. (27%) with maximum germination percentage 98.25.

The relative abundance of *Aspergillus flavus* (158.5%) were found maximum from seed lots of pea varieties and it appeared frequently in Ambika (33.25%) followed by Indira matar (31%) and KPMR 400 (29.25%). Other predominant mycoflora were *Alternaria* sp. (131.75%), *Chaetomium* sp. (108.25%), *Aspergillus niger* (66.75%), *Basidiophora* sp. (39.75%), *Curvularia* sp. (31.75%), *Aspergillus fumigatus* (24.75%), *Rhizopus* sp. (24%) and *Trichoderma* sp. (21.25%). *Penicillium* sp. were detected with least frequency (8.75%) and detected only in Paras variety.

In this method, local variety shows the highest frequency of mycoflora with lowest germination percentage while least frequency of mycoflora with highest germination percentage was recorded in Shubhra variety among all the varieties taken in the study. Pea varieties were found associated with *Aspergillus flavus* in high frequency and *Penicillium* sp. were found with least frequency.

Rauf (2000), Begum *et al.*, (2004), Verma *et al.*, (2004), Narayan *et al.*, (2013) and Ghangaokar *et al.*, (2013) also identified various seed borne mycoflora associated with pea in varying frequencies by blotter paper method. These findings are in agreement with the findings of present study, in which decreasing trend in seed germination and increasing trend in seed mycoflora were recorded. In other legumes also, standard blotter method appeared as useful in detecting seed borne mycoflora efficiently. Barua *et al.*, (2007), Ashwini *et al.*, (2014), Mohana *et al.*, (2015) and Pradhan (2017) reported in green gram; Dawar *et al.*, (2007), Ghangaokar *et al.*, (2013), Margaret *et al.*, (2013), Sontake *et al.*,

(2014), Mohana *et al.*, (2015), Kumar (2016) reported in chickpea and Patil *et al.*, (2012), Ghangaokar *et al.*, (2013), Pradhan (2014) and Chaudhary *et al.*, (2017) reported in pigeonpea

Agar plate method

Seeds of seven pea varieties were evaluated for associated seed borne mycoflora in agar plate method and data presented in table 2. Maximum frequency of mycoflora were recorded from seeds of local variety (131.25%) which include *Aspergillus flavus* (53.5%), *Aspergillus niger* (28.75%), *Basidiophora* sp. (5%), *Rhizopus* sp. (10.75%), *Rhizoctonia* sp. (14.25%) and *Fusarium* sp. (19%) with least germination (58.5%) followed by KPMR 400 seed lot (113%) with germination percentage 69.25. Frequencies of mycoflora recorded from the seeds of other pea varieties were Ambika (103.65%), IPFD10-12 (102.50%), Paras (97%) and Indira matar (81.75%) with varying germination percentage as 83.25, 84.5, 89.25, and 94, respectively. Least frequency of mycoflora were recorded from seeds of Shubhra variety (80%) with highest germination percentage -97.75.

Various mycoflora were detected from pea varieties by this method, in which relative abundance of *Aspergillus flavus* (250.5%) was recorded and it was most frequent in KPMR 400 (62.75%) and local variety (53.5%). This was followed by *Rhizoctonia* sp. (112.4%), *Rhizopus* sp. (94.5%), *Aspergillus niger* (69.75%), *Fusarium* sp. (60%), *Trichoderma* sp. (44.25%) and *Aspergillus fumigatus* (40.25%). *Basidiophora* sp. (37.5%) were found with least frequency in pea varieties.

It appears that among all pea varieties, local variety showed maximum frequency of mycoflora and lowest germination, while Shubhra variety showed minimum frequency of mycoflora with maximum germination.

Table.1 Detection of mycoflora associated with seeds of pea by Standard Blotter Method

Varieties	Germination (%)	Frequency of mycoflora associated (%)										Total frequency (%)
		<i>A.flavus</i>	<i>A.niger</i>	<i>A.fumigatus</i>	<i>Trichoderma sp.</i>	<i>Alternaria sp.</i>	<i>Curvularia sp.</i>	<i>Chaetomium sp.</i>	<i>Rhizopus sp.</i>	<i>Basidiophora sp.</i>	<i>Penicillium sp.</i>	
IPFD10-12	90.50	10.25	8.00	4.00	6.25	11.00	5.50	20.50	8.50	6.75	-	80.75
Paras	97.50	18.25	7.75	6.50	-	12.00	6.75	7.50	-	9.50	8.75	77.00
KPMR 400	84.50	29.25	9.25	-	15.00	39.25	-	4.75	-	-	-	97.50
Indira matar	85.00	31.00	-	3.75	-	22.50	19.5	-	-	11.00	-	87.75
Shubhra	98.25	15.50	18.50	4.50	-	-	-	27.00	-	-	-	65.50
Ambika	83.50	33.25	12.75	-	-	13.5	-	23.75	-	12.25	-	95.50
Local variety	75.76	21.00	10.50	6.00	-	33.50	-	24.75	15.5	-	-	111.25
Total mycoflora		158.50	66.75	24.75	21.25	131.75	31.75	108.25	24	39.75	8.75	

Table.2 Detection of mycoflora associated with seeds of pea by agar plate method

Varieties	Germination (%)	Frequency of mycoflora associated (%)								Total frequency
		<i>A. flavus</i>	<i>A. niger.</i>	<i>A. fumigatus</i>	<i>Trichoderma sp.</i>	<i>Basidiophora sp.</i>	<i>Rhizopus sp.</i>	<i>Rhizoctonia sp.</i>	<i>Fusarium sp.</i>	
IPFD 10-12	84.50	48.25	-	5.00	-	13.75	35.50	-	-	102.50
Paras	89.25	19.00	14.75	6.75	-	7.00	11.00	25.75	12.75	97.00
KPMR 400	69.25	62.75	-	28.50	-	11.75	-	10.00	-	113.00
Indira Matar	94.00	33.25	5.25	-	6.25	-	12.25	13.25	11.50	81.75
Shubhra	97.75	18.75	10.00	-	26.25	-	25.00	-	-	80.00
Ambika	83.25	15.00	11.00	-	11.75	-	-	49.15	16.75	103.65
Local Variety	58.50	53.50	28.75	-	-	5.00	10.75	14.25	19.00	131.25
Total mycoflora		250.5	69.75	40.25	44.25	37.50	94.50	112.4	60.00	

Table.3 (a) Detection of mycoflora associated with seeds of pea by roll paper towel method

Varieties	Germination (%)	Frequency of mycoflora associated (%)								Total Frequency
		<i>A. flavus</i>	<i>A. niger</i>	<i>A. umigatus</i>	<i>Trichoderma</i> sp.	<i>Alternaria</i> sp.	<i>Curvularia</i> sp.	<i>Chaetomium</i> sp.	<i>Rhizopus</i> sp.	
IPFD 10-20	93.98	11.66	5.33	6.66	1.66	37.00	15.33	-	1.66	79.30
Paras	96.98	19.66	6.33	-	8.00	-	17.00	11.66	12.00	74.65
KPMR 400	92.65	20.00	12.66	1.33	4.33	20.00	12.66	5.33	6.66	82.97
Indira matar	94.32	11.33	15.00	4.66	5.33	28.00	7.66	6.33	-	78.31
Shubhra	98.98	17.33	3.66	-	7.00	-	32.00	3.66	3.00	66.65
Ambika	91.31	23.66	8.00	-	15.33	2.00	24.33	10.33	-	83.65
Local variety	88.99	12.00	6.66	3.00	3.66	17.00	23.00	26.33	6.00	97.65
Total mycoflora		115.64	57.64	15.65	45.31	104	131.98	63.64	29.32	

Table.3 (b) Categorization of seeds and seedlings of pea varieties by roll paper towel method

Varieties	Normal Seedling			Total (A)	Abnormal seedling			Total (B)	Ungerminated seed			Total (C)	Total Seedling (A+B)
	Intact	Slight defect	Secondary infection		Damaged seedling	Deformed	Decayed		Hard seed	Fresh seed	Dead seed		
IPFD 10-12	2.66	7.66	76.00	86.32	-	3.33	4.33	7.66	-	5.00	1.00	6.00	93.98
Paras	6.33	2.66	72.00	80.99	3.66	4.00	8.33	15.99	-	-	3.66	3.66	96.98
KPMR 400	2.33	7.00	66.33	75.66	2.66	3.33	11.00	16.99	-	3.33	4.00	7.33	92.65
Indira matar	15.66	-	70.00	85.66	-	2.33	6.33	8.66	-	4.33	1.33	5.66	94.32
Shubhra	10.66	2.33	61.00	73.99	3.00	5.66	16.33	24.99	-	-	0.66	0.66	98.98
Ambika	7.66	2.66	67.00	77.32	-	6.33	7.66	13.99	-	4.00	3.66	7.66	91.31
Local variety	-	2.33	47.33	49.66	-	2.33	37.00	39.33	-	7.00	2.66	9.66	88.99

Table.4 Detection of mycoflora associated with pea seeds by deep freeze method

Varieties	Germination (%)	Frequency of mycoflora associated (%)					Total Frequency (%)
		<i>A. flavus</i>	<i>A. niger</i>	<i>Rhizopus</i> sp.	<i>Alternaria</i> sp.	<i>Curvularia</i> sp.	
IPFD 10-12	88.75	34.50	13.75	37.25	-	-	85.50
Paras	93.50	10.00	16.50	25.75	8.25	3.50	64.00
KPMR 400	76.25	25.75	-	41.25	37.50	-	104.50
Indira matar	89.50	16.00	11.25	38.50	-	-	65.75
Shubhra	97.75	13.50	6.25	25.50	-	-	45.25
Ambika	86.50	31.25	8.75	46.00	6.75	-	92.75
Local variety	55.25	35.75	30.00	37.50	7.50	31.00	141.75
Total mycoflora		166.75	86.50	251.75	60.00	34.50	

As compare to other mycoflora, *Aspergillus flavus* was most frequently associated mycoflora with pea varieties and *Basidiophora* sp. were least frequent in all pea varieties. *Alternaria* sp., *Curvularia* sp., *Chaetomium* sp. and *Penicillium* sp. were not detected in this method, which were detected in standard blotter paper method.

Grzelak (1973), Ali (1982), Nagerabi *et al.*, (2000), Begum *et al.*, (2004), Verma *et al.*, (2004) and Ozgonen *et al.*, (2011) also detected the seed borne mycoflora associated with pea seeds by agar plate method. Patil *et al.*, (2012), Pradhan (2014) and Chaudhary *et al.*, (2017) in pigeopea; Dawar *et al.*, (2007), Shaker *et al.*, (2010), Sontakke *et al.*, (2014), Mohana *et al.*, (2015), Trivedi *et al.*, (2015), Kumar (2016), Hirwani (2016) in chickpea and Rathod *et al.*, (2012) in various legumes screened the mycoflora by this method. Present study is in corroboration with the findings of above researcher.

Roll paper towel method

As per ISTA, seeds get germinated to form seedlings, categorised as normal seedlings and abnormal seedlings. Normal seedlings

include intact seedlings, seedlings with slight defect and seedlings with secondary infection; Abnormal seedlings include damaged, deformed and decayed seedlings. Whereas, ungerminated seeds include hard seeds, fresh seeds and dead seeds.

Seed lot of seven varieties of pea were examined for associated seed borne mycoflora in varying frequencies with normal seedling, abnormal seedling and ungerminated seeds by roll paper towel method. It was observed that presence of mycoflora may be the cause of abnormalities and failure in germination. In this method mycoflora were found associated with seeds and seedling of pea varieties. Maximum frequency of mycoflora were recorded [Table 3(a) and (b)] from local variety seed lot (97.65%) and mycoflora detected were *Aspergillus flavus* (12%), *Aspergillus niger* (6.66%), *Aspergillus fumigatus* (3%), *Trichoderma* sp. (3.66%), *Alternaria* sp.(17%), *Curvularia* sp. (23%), *Chaetomium* sp. (26.33%) and *Rhizopus* sp. (6%) and it showed least germination (88.99%) with normal seedling (49.66%) and abnormal seedling (39.33%) followed by frequency of mycoflora in Ambika (83.65%), KPMR 400 (82.97%), IPFD 10-12 (79.3%),

Indira matar (78.31%) and Paras (74.65%) and germination percentage recorded in all these pea varieties were 91.31, 92.65, 93.98, 94.32 and 96.98, respectively. Shubhra variety showed minimum frequency of mycoflora (66.65%) includes *Aspergillus flavus* (17.33%), *Aspergillus niger* (3.66%), *Trichoderma* sp. (7%), *Curvularia* sp. (32%), *Chaetomium* sp. (3.66%), *Rhizopus* sp. (3%) and it showed highest germination (98.98%) with normal seedling (73.99%), abnormal seedling (24.99%) and ungerminated seeds (0.66%).

In all varieties of pea, relative abundance of *Curvularia* sp. (131.98%) were recorded and it was detected most frequently from pea varieties Shubhra (32%), Ambika (24.33%) and local variety (23%) followed by *Aspergillus flavus* (115.64%), *Alternaria* sp. (104%), *Chaetomium* sp. (63.64%), *Aspergillus niger* (57.64%), *Trichoderma* sp. (45.31%) and *Rhizopus* sp. (29.32%). *Aspergillus fumigatus* (15.65%) were least frequent mycoflora recorded in pea varieties in roll paper towel method. Commonly occurring seed borne mycoflora found associated with seeds of chickpea and pigeonpea were reported by Singh *et al.*, (2014) and seed borne mycoflora of pigeonpea by Pradhan (2014) were in conformity with the findings of present study in which most common fungi in varying frequencies and their impact on germination were recorded.

Deep freeze method

Seed lots of pea varieties were evaluated for the associated seed borne mycoflora by using deep freeze method and data presented in table 4. Frequency of mycoflora associated were maximum in local variety (141.75%) and mycoflora detected as *Aspergillus flavus* (35.75%), *Aspergillus niger* (30%), *Rhizopus* sp. (37.5%), *Alternaria* sp. (7.5%) and

Curvularia sp. (31%) with lowest germination (55.25%). It was followed by KPMR 400 (104.5%), Ambika (92.75%), IPFD10-12 (85.5%), Paras (64%) and Indira matar (65.75%) with the germination percentage - 76.25, 86.5, 88.75, 93.5 and 89.5, respectively. Least frequency of mycoflora recorded from Shubhra variety (45.25%) and mycoflora detected as *Aspergillus flavus* (13.5%), *Aspergillus niger* (6.25%), *Rhizopus* sp. (25.5%) and highest germination (97.75%) was also recorded.

In this method, relative abundance of *Rhizopus* sp. (251.75%) was recorded, followed by *Aspergillus flavus* (166.75%), *Aspergillus niger* (86.5%) and *Alternaria* sp. (60%). Frequency of *Rhizopus* sp. were found maximum in Ambika variety (46%), followed by KPMR 400 (41.25%) and Indira matar (38.5%). Frequency of occurrence was least in *Curvularia* sp. (34.5%) and it was present only in local variety (31%) and Paras (3.5%) variety.

In this method mycoflora associated with seeds of pea varieties were observed maximum in local variety with lower germination percentage, while minimum frequency of mycoflora were observed from seeds of Shubhra variety with higher germination than the other varieties taken in the study. Dawar *et al.*, (2007) also detected seed borne mycoflora in chickpea seed by deep freeze method.

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