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Detection of Seed Borne Myco-Flora Associated with Chickpea

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ABSTRACT

The seed borne myco-flora of 10 varieties of chickpea, Vijay, Jaki-9218, ICCC-37, Digvijay, PKV Kabuli-2, Vihar, Virat, Saki-9516, AKGS-1 and Gujarat-1 collected from different location of Maharashtra were examined by blotter method ,agar plate method, deep freezing and 2-4 D method as recommended by ISTA. A total of six genera of fungi viz. Alternaria alternata, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Penicillium spp. Rhizopus spp., Curvullaria lunata and Rhizoctonia bataticola were reported. Among them Aspergillus niger, Aspergillus flavus, Fusarium oxysporum and Rhizopus spp. were predominant to all seed samples. Amongst the methods used for detection of seed borne fungi, the blotter paper method is more effective followed by agar plate method, deep freeze method and 2, 4-D method.

Key words: *Chickpea, seed borne mycoflora, blotter paper method, agar plate method, deep freeze method, 2, 4-D method.*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a most versatile crop, is grown in all three season in the most part of the world and is a primary source of proteins. It is commonly known as *channa*, gram, garbanzo bean etc. Its center of origin is in Eastern Mediterranean. The other major chickpea producing countries include Pakistan, Turkey, Iran, Myanmar, Australia, Ethiopia, Canada, Mexico and Iraq. The number of diseases recorded on the chickpea has been increased and those which were formally of little importance have now become more significant. There are more than 50 pathogens known to attack chickpea of which about 40 are of economic important. Many factors is involved in reducing chickpea seed quality but a combination of susceptibility to pathogenic fungi, environmental condition that favour disease development and field detoriation of seed. Moreover channa seeds are rich in protein contain and therefore easily infected by several field and storage fungi which not only affect seed quality but also emergence in field. Shahnazdawa *et al*[2]., detected several fungal species belonging to many genera from cultivars of chickpea seed-borne fungi of chickpea have been also reported by various workers from time to time. Rathod *et al.*[9], and Narayan *et al*[8].

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Chickpea seeds are known to carry several pathogens which cause heavy yield losses and seed vigour, cause transmission of disease in growing plant and affect dal and channa quality. Hence the present study on detection of seed-borne mycoflora of chickpea was conducted to know the seed mycoflora associated with the chickpea seed.

MATERIAL AND METHODS

Collection of seed samples of chickpea

The seed sample of different cultivars of chickpea *viz*., Vijay, Jaki-9218, ICCC-37, Digvijay, PKV Kabuli-2, Vihar, Virat, Saki-9516, AKGS-1, Gujarat-1 were collected from various locations of Maharashtra.

Blotter Paper Method

Standard blotter method was used for the detection of seed borne fungi of chickpea. The 200 seeds of each variety were sown on three layers of pre-soaked moist blotter paper having 9 cm diameter. In each plate 10 seed were arranged, 9 seeds in the outer ring and one in the center of plastic plates.

Agar Plate Method

Agar plate method is preferred mostly in plant pathological studies as it provides nutrients rich substrate for development of mycelial growth and sporulation of pathogen on seed, particularly for slow growing fungi, PDA medium was autoclaved at 1.04 kg/cm^2 for 15 minute and warm medium (45^0C) approximately 20 ml per plate was poured. On solidification 200 seeds were plated, each plate containing 10 seeds.

Deep Freeze Method

This method allows better growth of certain fungi as the imbibed seeds on moist blotters are killed by deep freezing and the enclosed nutrients in seed are utilized by fungi. Two hundred seeds were plated as in blotter method and incubated for 24 hr at room temperature (27 ± 2^0C) then the plated seeds were deep frozen at - 20 for 24 hr

2, 4 - D Method[5]

2,4-D, is a herbicide retards seed germination and seedlings growth due to which the seeds are not displaced and the examination of fungi becomes easy, the blotter were soaked in 0.1 per cent 2,4-D suspension and then placed in plates. Two hundred seeds were incubated for seven days as in blotter method. The plated seeds were then incubated at 27 ± 2^0C, under alternate cycle of 12 hr light and 12hr darkness for seven days by using two 40W white fluorescent tubes. After seven days of incubation, seeds were examined under stereoscopic microscope by using a magnification of 6X to 50X. Research microscope was also used to confirm the identification of fungi based on morphological characters given in standard mycological books.

RESULT AND DISCUSSION

The seed sample of different cultivars of chickpea viz., Vijay, Jaki-9218, ICCC-37, Digvijay, PKV Kabuli-2, Vihar, Virat, Saki-9516, AKGS-1, and Gujarat-1 were collected from various locations of Maharashtra and tested for seed borne mycoflora by different methods. The seed samples of different cultivars of chickpea recorded eight fungi (Table 1) viz., *Alternaria alternata*, the infection ranges from 2 to 9.2%, *Aspergillus flavus* 18.1 to 28.4%, *A. niger* 26.8 to 34.4%, *Fusarium oxysporum* 19.5 to 26.3%, *Penicillium spp.* 0 to 12.1%, and *Rhizopus spp.* 19.5 to 31.1%. *Curvullaria lunata* 0 to 3%, *Rhizoctonia bataticola* 0 to 5.5%.The seed sample of variety Digvijay recorded highest association of total fungi 135.5 per cent. Maximum association recorded, *Aspergillus niger* (32.7%), followed by *Rhizopus spp.* (31.1%), *Aspergillus flavus* (28.4%),*Fusarium oxysporum* (23.1%) and minimum was observed in Rhizoctonia bataticola (5%) followed by *Alternaria alternata* (7.2%), *Penicillium spp.* (8%) and *Curvullaria lunata* was recorded as absent. The seed sample of variety Jaki-9218 recorded lowest association of total fungi 89.3 per cent. Maximum association recorded, *Aspergillus niger* (30.2%), followed by *Rhizopus spp.* (31.1%), *Fusarium oxysporum* (19.5%) *Aspergillus* flavus (18.1%) and minimum was observed in *Alternaria alternata* (2%), and *Rhizoctonia bataticola, Curvullaria lunata, Penicillium spp.* was recorded as absent. The

highest total fungi (135.5%) was recorded in Digvijay samples followed by Virat (135.4%), Vihar (129.6%), Vijay (122.1%), Gujarat-1 (114.1%), and minimum recorded in Jaki-9218 (89.3%) followed by PKV Kabuli-2 (100.5%), AKGS-1 (105.9%), Saki-9516 (106.8%) and ICCC-37 (112.4%).The *Aspergillus niger, Rhizopus spp., Aspergillus flavus, Fusarium oxysporum, Alternaria alternata* was predominant fungi in all the samples. Similar type of results is given by Muthuraj *et al*[7]., Shahnazdawa *et al*.[2]. (2007) and Kandhare A.S[3].

In Agar plate method (Table-2), different cultivars of chickpea recorded eight fungi viz., *Alternaria alternata,* the infection from 1.7 to 7.5%, *Aspergillus flavus* 16.2 to 26.4%, *A. niger* 24.6 to 32.5%, *Fusarium oxysporum* 17.6 to 22.7%, *Penicillium spp.* 0 to 10.3%, and *Rhizopus spp.* 11.5 to 27.4%. *C. lunata* 0 to 2%, *R. bataticola* 0 to 4.1%.The seed sample of variety Virat recorded highest association of total fungi 122.9 per cent. Maximum association recorded, *Aspergillus niger* (32.5%), followed by *Rhizopus spp.* (27.4%), *Aspergillus flavus* (23.2%), *Fusarium oxysporum* (22.7%) and minimum was observed in *Alternaria alternata* (4.8%) followed by *R. bataticola* (2%), *Penicillium spp.* (10.3%) and *C. lunata* was recorded as absent. The seed sample of variety Jaki-9218 recorded lowest association of total fungi 83.3 per cent. Maximum association recorded, *Aspergillus niger* (26.7%), followed by *Fusarium oxysporum* (24.5%), *Aspergillus flavus* (16.2%), *Rhizopus spp.* (14.2%), and minimum was observed in *Alternaria alternata* (1.7%), and *R. bataticola, C. lunata, Penicillium spp.* was recorded as absent. The frequency of *Aspergillus niger* was found maximum with Virat, Vijay, Digvijay. Similarly prominent association of *A. flavus* found in PKV Kabuli-1, Saki-9516 Moderate association of *Alternaria alternata, Rhizopus spp.* and minimum association *R. bataticola, C. lunata, Penicillium spp.* in overall seed samples. Among the storage fungi *A. flavus, A. niger* and *Rhizopus spp.* was observed more on all seed sample. Similar results were observed by Muthuraj *et al*[7]., Shahnazdawa *et al*[2]., and Kandhare A.S[3].

In deep freeze method (Table 3) seed samples of different cultivars of chickpea recorded eight fungi viz., *Alternaria alternata,* the infection from 0 to 6%, *Aspergillus flavus* 11.1 to 23.5%, *A. niger* 21.3 to 30.4%, *Fusarium oxysporum* 15.3 to 22.2%, *Penicillium spp.* 0 to 6.5%, and *Rhizopus spp.* 12.5 to 24.7%. *Curvularia* lunata 0 to 2%, *R. bataticola* 0 to 2.1%. The seed sample of variety Vihar recorded highest association of total fungi 105.7 per cent. Maximum association recorded, *Aspergillus niger* (30.5%), followed by *Rhizopus spp.* (23.2%), *Aspergillus flavus* (21.5%), *Fusarium oxysporum* (20.6%) and minimum was observed in *R. bataticola* (1%) followed by *Alternaria alternata* (3.5%), *Penicillium spp.* (5.5%) and *C. lunata* was recorded as absent, but whereas frequency of it was (2%) in variety ICCC-37.The seed sample of variety Jaki-9218 recorded lowest association of total fungi 65.4 per cent. Maximum association recorded, *Aspergillus niger* (21.3%), followed by *Fusarium oxysporum* (20.5%), *Rhizopus spp.* (12.5%), and minimum was observed in *Aspergillus flavus* (11.1%), and *A. alternata, R. bataticola, C. lunata,* and *Penicillium spp.* was recorded as absent. The fungi *Aspergillus niger, Rhizopus spp, Aspergillus flavus, Fusarium oxysporum* was predominant fungi in all the samples, similar type of results are given by Rathod *et al*[9]. and Kandhare A.S[3].

In 2, 4-D method (Table 4) the seed samples of different cultivars of chickpea recorded eight fungi *viz., Alternaria alternata,* the infection from 0 to 6%, *Aspergillus flavus* 9.5 to 16.1%, *A. niger* 12.5 to 18.5%, *Fusarium oxysporum* 10.5 to 16.2%, *Penicillium spp.* 0 to 2%, and *Rhizopus spp.* 6.5 to 11.1%. *C. lunata* 0 to 2%, *R. bataticola* 0 to 2%. The seed sample of variety Virat recorded highest association of total fungi 68.5 per cent. Maximum association recorded, *Aspergillus niger* (18.5%), followed by *Fusarium oxysporum* (16.2%), *Aspergillus flavus* (16.1%), *Rhizopus spp.* (10.2%), and minimum was observed in *R. bataticola* (1%)

followed by *Alternaria alternata* (3.5%), *Penicillium spp.* (2%) and *Curvularia lunata* (1%). The seed sample of variety PKV Kabuli-1 recorded lowest association of total fungi 39.9 per cent. Maximum association recorded, *Aspergillus niger* (12.2%), followed by *Fusarium oxysporum* (11.5%), *Aspergillus flavus* (9.5%), and minimum was observed in *Rhizopus spp.* (6.4%), and *A. alternata, Penicillium spp., R. bataticola , C. lunata* was recorded as absent. However, *Aspergillus niger, Rhizopus spp, Aspergillus flavus, Fusarium oxysporum* was predominant fungi in all the samples, similar type of results are given by Muthuraj *et al*[7]. and Kandhare A.S[3].

The data from the (Table -5) revealed that, Blotter Paper Method was found best method (115.2%) for testing of seed borne fungi followed by Agar Plate Method (104.87%), Deep Freeze Method (88.64%) and 2, 4-D Method (54.53%). These findings are similar to the results obtained by Khan *et al*[4]., Arya *et al*[1]., Mogle and Maske[6]. Among all the methods tested *Aspergillus niger* 15.67 to 31.44% was found more predominant followed by *Rhizopus spp.* 8.9 to 24.11% *Aspergillus flavus* 12.78 to 31.44%, *Fusarium oxysporum* 12.98 to 22.25% and minimum association recorded of *Alternaria alternata* 2.2 to 4.92% followed by *Penicillium spp.* 0.7 to 4.54%. *Rhizoctonia bataticola* 0.5 to 2.11% and *Curvularia lunata* 0.8 to 1.15%.

Table 1.The frequency of seed borne fungi associated with Chickpea seeds (Blotter Paper Method)

Seed borne fungi	Per cent association of seed borne fungi (%)									
	Jaki-9218	**Vijay**	**Digvijay**	**Saki-9516**	**PKV Kabuli-2**	**AKGS-1**	**Vihar**	**Virat**	**ICCC-37**	**Gujarat-1**
A. alternata	2	4.5	7.2	3	4	4	9.2	6.3	5	4
A. flavus	18.1	26.3	28.4	26.1	23.9	22.3	28.1	25.2	25.7	22.3
C. lunata	-	3	-	-	2	1.5	2	-	3	-
A. niger	30.2	33.4	32.7	28.2	32.2	31.8	32.5	34.4	32.2	26.8
F. oxysporum	19.5	22.6	23.1	22.3	21.4	21.6	26.3	25.1	20.7	19.9
R. bataticola	-	2	5	-	2.5	-	4	2.1	-	5.5
Rhizopus spp.	19.5	30.3	31.1	27.2	19.5	13.5	27.5	30.2	25.8	26.5
Penicillium spp	-	-	8	-	5	11.2	-	12.1	-	9.1
Total fungi	89.3	122.1	135.5	106.8	100.5	105.9	129.6	135.4	112.4	114.1

Table 2. The frequency of seed borne fungi associated with Chickpea seeds (Agar Pate Method)

Seed borne fungi	Per cent association of seed borne fungi (%)									
	Jaki-9218	**Vijay**	**Digvijay**	**Saki-9516**	**PKV Kabuli-2**	**AKGS-1**	**Vihar**	**Virat**	**ICCC-37**	**Gujarat-1**
A. alternata	1.7	2.5	5.1	2	3.1	2	7.5	4.8	3.5	2
A. flavus	16.2	24.1	26.4	23.6	20.3	21.4	25.3	23.2	23.5	20.1
C. lunata	-	2	-	1	2	1.5	1	-	2	-
A. niger	26.7	30.2	31.1	26.2	30.8	29.5	30.5	32.5	30.2	24.6
F. oxysporum	24.5	22.6	20.3	20.1	19.5	18.4	24.8	22.7	18.4	17.6
R. bataticola	-	1	4.1	-	2.5	-	2	2	-	3
Rhizopus spp.	14.2	27.5	28.7	25.3	19.5	11.5	25.8	27.4	23.1	24.8
Penicillium spp	-	-	6.2	-	4	9.6	-	10.3	-	7.2
Total fungi	83.3	109.9	121.9	98.2	101.7	93.9	116.9	122.9	100.7	99.3

Table 3. The frequency of seed borne fungi associated with Chickpea seeds (Deep Freeze Method)

Seed borne fungi	Per cent association of seed borne fungi (%)									
	Jaki-9218	**Vijay**	**Digvijay**	**Saki-9516**	**PKV Kabuli-2**	**AKGS-1**	**Vihar**	**Virat**	**ICCC-37**	**Gujarat-1**
A. alternata	-	2.5	4	2	3	1	3.5	6	3	1.5
A. flavus	11.1	19.5	22.1	21.5	18.5	19.5	21.5	23.5	19.6	18.5
C. lunata	-	-	-	-	1	1	-	1	2	-
A. niger	21.3	22.2	26.5	23.4	25.5	26.4	30.4	28.5	27.5	22.5
F. oxysporum	20.5	20.3	20.3	18.7	15.3	16.4	20.6	22.2	16.5	15.6
R. bataticola	-	-	2.1	-	1	-	1	2	-	2
Rhizopus spp.	12.5	17.6	24.7	23.7	15.5	10.5	23.2	16.5	20.5	21.4
Penicillium spp.	-	-	4.2	-	3	6.5	5.5	-	-	5.6
Total fungi	65.4	82.1	103.9	89.3	82.8	81.3	105.7	99.7	89.1	87.1

Table 4. The frequency of seed borne fungi associated with Chickpea seeds (2, 4-D Method)

Seed borne fungi	Per cent association of seed borne fungi (%)									
	Jaki-9218	**Vijay**	**Digvijay**	**Saki - 9516**	**PKV Kabuli-2**	**AKGS-1**	**Vihar**	**Virat**	**ICCC-37**	**Gujarat-1**
A. alternata	3	1	2	1	-	1	6	3.5	3	1.5
A. flavus	13.2	8.5	12.5	11.1	9.5	14.7	15.2	16.1	14.5	12.5
C. lunata	1	-	2	-	-	1	1	1	2	-
A. niger	16.3	14.5	15.5	17.3	12.5	15.2	15.5	18.5	17.1	14.3
F. oxysporum	13.4	12.4	14.5	12.5	11.5	10.5	15.5	16.2	12.2	11.1
R. bataticola	-	1	1	-	-	-	2	1	-	-
Rhizopus spp.	8.2	11.1	10.2	9.5	6.4	7.3	10	10.2	8.6	7.5
Penicillium spp	1	-	2	-	-	-	-	2	-	2
Total fungi	56.1	48.5	59.7	51.4	39.9	49.7	65.2	68.5	57.4	48.9

Table 5. Comparative evaluation of different incubation methods for association of seed-borne fungi in chickpea

Seed-borne fungi	Per cent association of seed borne fungi (%)			
	Blotter Paper Method	**Agar Plate Method**	**Deep freeze Method**	**2,4-D Method**
A. alternata	4.92	3.42	2.65	2.2
A. flavus	24.64	22.41	19.53	12.78
C. lunata	1.15	0.95	0.5	0.8
A. niger	31.44	29.23	25.42	15.67
F. oxysporum	22.25	20.89	18.64	12.98
R. bataticola	2.11	1.46	0.81	0.5
Rhizopus spp.	24.11	22.78	18.61	8.9
Penicillium spp	4.54	3.73	2.48	0.7
Total fungi	115.2	104.87	88.64	54.53

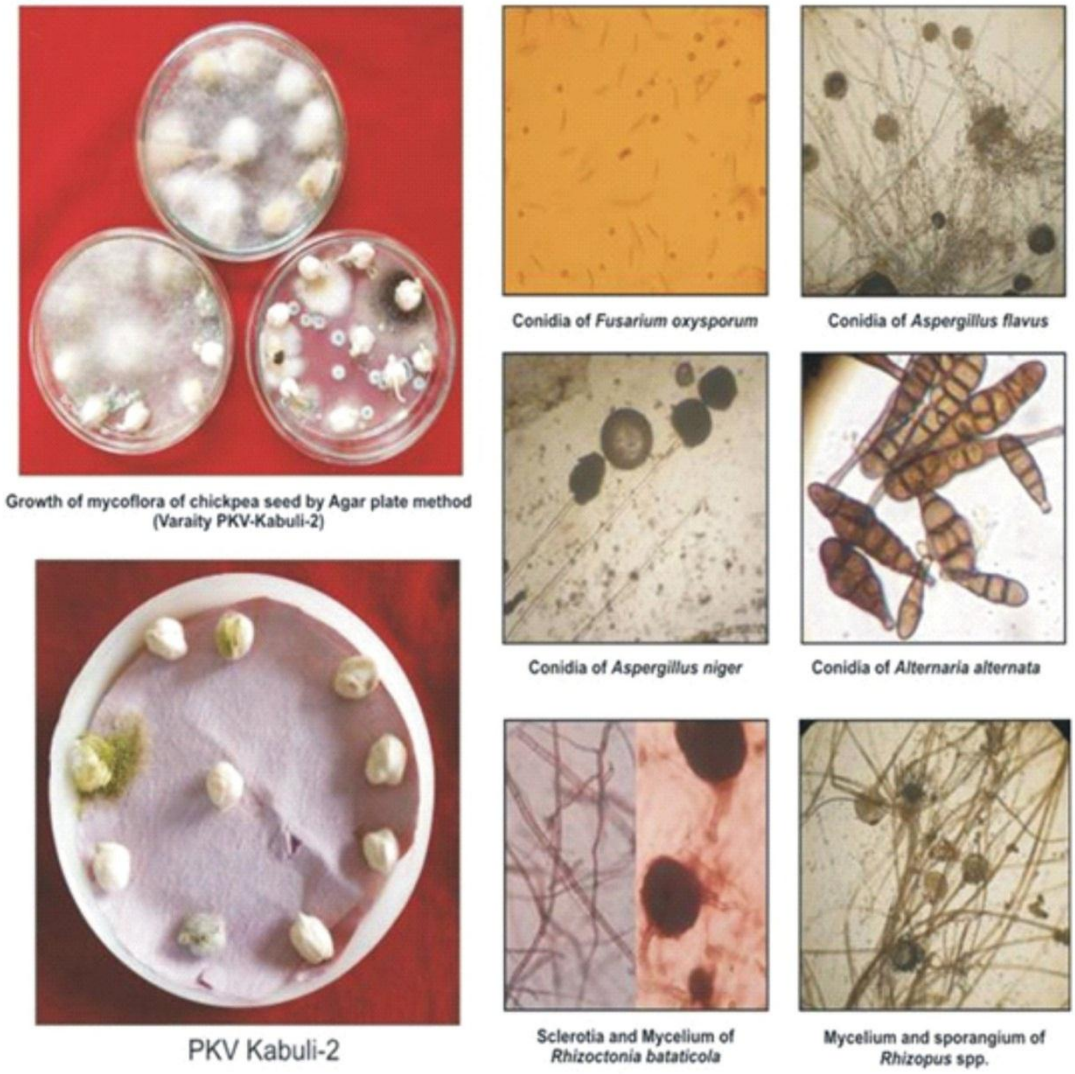

Plate- 1 Showing agar plate method, Blotter plate method and different genera of seed born fungi observed on Chick pea seed

CONCLUSION

Amongst the four method used for testing of seed of chickpea *viz*., Vijay, Jaki-9218, ICCC-37, Digvijay, PKV Kabuli-2, Vihar, Virat, Saki-9516, AKGS-1, Gujarat-1 , the blotter Paper Method was found best method (115.2%) for testing of seed borne fungi followed by Agar Plate Method (104.87%), Deep Freeze Method (88.64%) and 2, 4-D Method (54.53%). Eight fungi were reported *viz, Alternaria alternata, Aspergillus flavus , Aspergillus niger, Fusarium oxysporum, Penicillium spp. Rhizopus spp., Curvullaria lunata and Rhizoctonia bataticola*. Amongst them *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum* and *Rhizopus spp.* were predominant to all seed samples. According to location Akola recorded highest association of total fungi followed by Aurangabad and Ahmednagar.

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