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Symptomatology, association and pathogenicity of seed Mycoflora in green gram

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Abstract
Greengram variety seeds viz., GM-3, GM-4 and K-851 were collected and studied for the symptomatology, association and pathogenecity of seed mycoflora associated with the seeds. Four types of broad category of symptoms *viz.*, bold healthy, bold discoloured, shrivelled and small seeds were observed and categorized primarily in all the seed lots. The observations on the specific symptoms on the broad categorized symptoms revealed the variety of symptoms *viz.*, seeds having healthy green, apparentely green, mosaic, brown lesion, black lesion, red to dark red spot and whole brownish colour. Study on the confirmation of the fungi associated with specific symptoms revealed the association and presence of specific fungi on each type of seed *viz.*, *Colletotrichum* sp. Was found associated with red to dark red spots while *Alternaria* sp. with black lesions and *Macrophomina* sp.with brown lesions. Presence of sterile unidentified fungi were associated with mixture of yellow and green coloured pattern on the greengram seeds, while a group of seed infecting mycoflora looked responsible for development of whole brownish discoloration. In all nine different fungi *viz.*, *Macrophomina phaseolina, Alternaria alternata, Colletotrichum capsici, Nigrospora spherica, Aspergillus* sp., *Rhizopus* sp., *Chaetomium* sp., sterile aseptate and sterile septate fungus were found associated with the infected seeds of green gram. A single or mixtures of fungi were also proved pathogenic by causing loss in seed germination as well as seed to seedling transmission on seed inoculation method.

Keywords: Greengram, seed mycoflora, pathogenecity

Introduction
Greengard [*Vigna radiata* (L.) Wilczek] is one of the important pulse crop grown extensively in Gujarat. It suffers from a numbers of seed borne fungal diseases especially during *Kharif* season in south Gujarat with the prevalence of congenial weather conditions throughout the crop season. Leaf spot, leaf blight and anthracnose and pod moulding are typical diseases which were commonly observed in field of usually grown greengram varieties *viz*., GM-3, GM-4 and K-851 in farmer's fields of south Gujarat. The seeds harvested thus contain infected seed with group of mycoflora. The mycoflora is responsible for carrying the seed borne diseases endemically. Thus, it is one of the important constraints in greengram production especially during *Kharif* season in south Gujarat and no work related to this carried out in the past. Thus the present investigation on the studies of symptomatology, association and pathogenicity of seed mycoflora in green gram was carried out.

Materials and methods
Three greengram variety seeds *viz.*, GM-3, GM-4 and K-851 were collected from Pulse Research station, NAU, Navsari during *Late kharif* 2010. These were separately subjected to observe and describe the broad and specific symptoms by using magnifying hand lens and stereoscopic microscope. Symptomatically vitiated seeds were also subjected to isolation (Moist blotter), purification (Single colony isolation) and identification (ITCC, New Delhi) of fungi associated with it. The pathogenecity was proved by seed inoculation method (using GM-3 seeds) with isolated individual and mixture of all fungi under pot study. The observations on the possibility of the cause of the pre-emergence mortality due to seed infecting fungi in respective treatment was taken by observing the ungerminated seeds after dugging out from the pots through naked eye or microscopic observations. The undiseased and diseased (rotted) seeds were calculated from the dugged out ungerminated seeds. The percentage of undiseased and diseased seeds were recorded at 10 days of sowing. Percent undiseased ungerminated seed, Percent pre-emergence mortality, Percent post- emergence mortality, Percent germination are calculated by using (F1-F4) formulae.

F1	% undiseased ungerminated seed	=	No. of undiseased un-germinated seeds / pot / No. of sown seeds/ pot	x 100
F2	% pre-emergence mortality	=	No. of diseased un-germinated seeds/ pot / No. of sown seeds/ pot	x 100
F3	% post- emergence mortality	=	No. of diseased died seedlings / pot / No. of total plants/ pot	x 100
F4	% germination	=	No. of normal seedlings / pot / No. of total plants/ pot	x 100

The development of symptoms on normal ungerminated seeds or seedlings and diseased seeds or seedlings were taken at 10 Days after sowing while symptoms on the remaining plants were also observed in the individual pot at 15 days regular interval starting after 10 days of sowing to 25 and 45 days after planting and noted as "+"=presence and "-"= absence. The isolation and confirmation of the inoculated pathogen was carried out from the symptoms developed on seeds/seedlings such as ungerminated seeds, seed rot, seedling necrosis, seedling rot/ damping off, lesions on the cotyledonary leaves, leaf spot, leaf blight and root rot developed at regular interval to prove the pathogencity and seed to seedling transmission of the respective pathogenic fungi. (Elwakil and Ghoneem, 1991) [3].

Results and Discussion

Symptomatology

Mainly four types of broad symptoms *viz*., bold healthy, bold discolored, shrivelled and small seeds were observed in the collected seeds of all the three varieties *i.e.* GM-3, GM-4 and K-851 (Table-1). Only single type of specific symptom like bold green healthy seeds was observed in bold healthy seed lot, five types of specific symptoms *viz.*, mosaic, brown lesion, black lesion, red to dark red spot and whole brownish coloured seeds were observed in bold discoloured seeds, six types of specific symptoms *viz.*, apparently green, mosaic, brown lesion, black lesion, red to dark red spot, and whole brownish coloured seeds in the shrivelled seeds and only apparently green seeds were observed in small seeds.

The present results are more or less similar with earlier workers (Agarwal *et al.* 1971; Suryanarayana, 1978; Saxena and Sinha, 1979; Patil *et al.* 1990; Patel, 2003 and Tandel, 2004) [1, 9, 7, 5, 4, 10]. The various symptoms appeared on the greengram seeds can be attributed due to the deteriorating effects induced by one or few seed-infecting fungi along with prevailing atmospheric conditions in the area or in the field during pod developing stage and harvesting time etc. The main source of the infection and development of symptoms on the seed might be due to the infected pods which were yielded from the severely infected fields by number of fungal diseases such as leaf spot (*A. alternata*), leaf blight (*M. phaseolina*) as investigated earlier by Patel (2003) [4] and Tandel (2004) [10] from south Gujarat area. Interestingly, small seeds without discolouration than that of bold discoloured and shrivelled seeds were also observed along with the bold healthy seeds while studying the symptomatology of greengram. It indicated that such seeds were produced from the fully developed small pods which might have yielded due to the indirect effect of seed borne diseases and ultimately reduction in photosynthesis. However, symptoms like discoloured and whole brownish coloured seeds were absent in this category indicated low infection of seed mycoflora to these categorized seeds. This was in line with the results of Bhale (2011) [2] who described the occurance of symptoms like seed size reduction due to seed infecting fungi in numbers of crops. Small healthy seed symptom in greengram recorded here is for the first time.

Isolation

Study on the confirmation by the isolation of the fungi associated with various types of symptoms revealed that red to dark red spots on the greengram seeds found to be due to *Colletotrichum* sp., while black lesions was due to *Alternaria* sp. and brown lesions on the seed occurred due to *Macrophomina* sp. The development of mixture of yellow and green coloured pattern on greengram seeds resulted in presence of sterile unidentified fungi, while a group of seed infecting mycoflora looked responsible for development of whole brownish coloured discolouration (Table-1). The isolation of seed mycoflora from the seed samples revealed the association of nine different fungi and were identified as *Macrophomina phaseolina* (Tassi) Goid (I.T.C.C. No.7811.10), *Alternaria alternata* (Fr.) Keissler (I.T.C.C. No. 7820.10), *Colletotrichum capsici* (Syd.) Butler & Bisby (I.T.C.C. No. 7813.10), *Nigrospora sphaerica* E. W. Masson (I.T.C.C. No. 7819.10), *Chaetomium* sp. (I.T.C.C. No. 7815.10), *Aspergillus* sp. (I.T.C.C. No. 7818.10), *Rhizopus oryzae* Went & Prins. Geerl. (I.T.C.C. No. 7817.10), sterile aseptate (I.T.C.C. No. 7814.10) and sterile septate fungus (I.T.C.C. No. 7816.10).

The present results were more or less similar to that of earlier workers who reported production of blackening and mummification of seed by *A. alternata* (Patel, 2003), production of brown coloured lesions covering with fungal growth and shrivelling of seeds by *M. phaseolina* (Tandel, 2004) [10] and production of brown coloured spots on seeds by *Colletotrichum* sp. (Suryanarayana, 1978) [9]. Moreover, the association of few to more numbers of fungi *viz*., *Alternaria longissima, Chaetomium* sp., *C. lindemuthianum, C. truncatum, M. phaseolina* etc with the discoloured seed of greengram was also reported earlier by Agarwal *et al.* (1971). Thus, different symptom categories of seeds revealed preferential and specific trend of fungal association and various fungi induced specific seed abnormality and symptoms on greengram seeds.

Pathogenecity

The results of the pathogenecity revealed that undiseased ungerminated seeds were found only in healthy seed sown pots (10.33%). There was not any single ungerminated seeds which was undiseased in all other treatments inoculated with the fungal pathogens. Per cent pre emergence mortality was found significantly higher in the seeds inoculated with mixture of all fungi (64.22%) as compared to the rest. Next higher pre-emegence mortality was recorded in *M. phaseolina* (31.11%). Moderate pre-emergence mortality was observed in *A. alternata* (27.66%) followed by *C. capsici* (23.33%), *Aspergillus* sp. (16.77%). *N. sphaerica* (14.11%), *R. oryzae* (13.22%) and *Chaetomium* sp. (12.22%). The lowest pre emergence mortality was observed in the seeds inoculated with sterile septate fungi (10.11%) and sterile aseptate fungi (10.10%), whereas pre emergence mortality due to any fungal

infection was not observed in the healthy seeds. Per cent post-emergence mortality was found significantly higher in the seeds inoculated with mixture of all fungi (28.74%) as compared to the rest. Next higher post-emergence mortality was recorded in *M. phaseolina* (25.11%). Moderate post-emergence mortality was observed in *C. capsici* (20.11%) followed by *A. alternata* (19.22%), *Aspergillus* sp. (13.11%), *N. sphaerica* (10.11%), *R. oryzae* (10.11%) and sterile septate fungi (9.11%). It was the lowest in sterile aseptate fungi (8.22%), and *Chaetomium* sp. (8.11%). Post emergence mortality due to any of the fungal infection was not observed in the healthy seeds. Significantly lower per cent seed germination was observed in the seed inoculated with mixture of all fungi (7.21%) as compared to the rest. It was also very poor in the seeds inoculated with *M.phaseolina* (43.77%). Moderate seed germination was observed in *A. alternata* (53.11%) followed by *C. capsici* (56.56%). Comparatively higher seed germination was observed in the seeds inoculated with aseptate sterile mycelium (82.12%) followed by septate sterile mycelium (81.10%), *Chaetomium* sp. (80.13%), *R. oryzae* (77.22%), *N. sphaerica* (75.77%) and *Aspergillus* sp. (70.11%). The highest seed germination was recorded in the healthy seeds (89.66%).Maximum seed germination (91.95%) was reduced in the seeds inoculated with mixture of all fungi. *M. phaseolina* also caused comparatively more effect on germination (51.18%). The loss in germination in *A. alternata* (40.77%) and *C. capsici* (36.48%) were medium while it was very less (8.41-21.80%) in rest of the fungal inoculated seeds. Study on the symptom development on seed or seedlings (Table-4.3) revealed that at 10th day of sowing symptoms like seed rot, seedling necrosis, lesions of brown to red colour on the cotyledonary leaves, damping of off seedlings and syndrome of all these symptoms were observed in the seeds inoculated with mixture of all the fungi as well as in individually inoculated seeds with *M. phaseolina, A. alternata* and *C. capsici.* Whereas, seed rot, seedling necrosis and damping off of emerged seedling were observed in the seeds inoculated with *Aspergillus* sp., seed rot and damping off of seedling symptoms were common in all the other fungi *viz*., *N. sphaerica*, *R. oryzae, Chaetomium* sp., sterile aseptate and septate fungi. Undiseased ungerminated seeds, healthy seedlings and healthy plants were noticed in the pots sown with healthy seeds. Leaf spot symptom was observed at 25 days after sowing in the plants in which *M. phaseolina* was inoculated while at later stage leaf blight was also noticed. Leaf spot symptom was recorded in *A. alternata* while anthracnose symptom was recorded in *C. capsici* inoculated plants. Leaf spot at initial stage and leaf blight as well as root rot at later stage were recorded in the seeds inoculated with mixture of all fungi. The plants were totally free of any disease in case of the pots in which healthy seeds were sown. Moreover, the isolation carried out from the symptoms developed on seed, seedlings or on plants viz., seedling rot, seedling necrosis, lesions of brown to red colour on the cotyledonary leaves, leaf spot, leaf blight and root rot yielded similar fungal association as that of inoculated individually on surface sterilized seeds. Thus, confirmed the pathogencity and seed to seedling transmission of inoculated fungi individually.

The same results on loss of seed germination and production of various symptoms after inoculation of various seed mycoflora was noticed earlier in greengram by Suhag (1975); Saxena and Sinha (1979) [87] and Rahman *et al.* (1999) [6] while proving the pathogenicity. Moreover, higher seedling mortality induced by *M. phaseolina*, *A.alternata* and *Aspergillus sp.* individually in greengram seeds was also noticed earlier by Patil *et al.* (1990) [5]. This is the first time report where leaf spot, leaf blight and root rot symptoms can be produced by seed inoculation with various seed mycoflora in greengram. Interestingly undiseased ungerminated seeds were obtained in the pots sown with healthy seeds found responsible for less germination. They may be due to the other reasons such as sowing of immature seeds, insect infested seeds or seeds with the latent infection by other type of pathogens than that of fungi.

Thus, mixture of all the fungi and three fungi individually *viz., M. phaseolina, A. alternata* and *C. capsici* not only proved to be pathogenic for the reduction of seed germination but also proved to be act as a primary source of infection of various diseases by transmitting the pathogen inoculum from seed to seedling. They proved as seed borne in nature causing leaf spot, leaf blight, anthracnose and root rot in greengram. The greengram seed infecting fungi individually, in few or in group were proved to be pathogenic not only to reduce seed germination but also to act as a primary source of infection for seed and soil borne field diseases by inducing a variety of disease symptoms at diverse period of time.

Table 1: Broad and specific symptomatology of collected seed samples of greengram with associated mycoflora.

Broad symptoms	Specific symptoms	M	A	C	N	Asp.	R	Cha.	St -I	St –II
Bold healthy seeds	Bold, green coloured	-	-	-	-	-	-	-	-	-
Bold discoloured	Mosaic (mixture of yellow and green) on seed	-	-	-	-	-	-	-	+	+
	Seeds with brown lesion	+	-	-	-	-	-	-	-	-
	Seed with black lesion	-	+	-	-	-	-	-	-	-
	Seed with red to dark red spot	-	-	+	-	-	-	-	-	-
	Whole brownish seed	+	+	+	+	+	+	+	+	+
Shrivelled seed having	Apparently green	-	-	-	-	-	-	-	-	-
	Mosaic (mixture of yellow and green) on seed	-	-	-	-	-	-	-	+	+
	Seeds with brown lesion	+	-	-	-	-	-	-	-	-
	Seed with black lesion	-	+	-	-	-	-	-	-	-
	Seed with red to dark red spot	-	-	+	-	-	-	-	-	-
	Whole brownish seed	+	+	+	+	+	+	+	+	+
Small seeds	Apparently healthy green	-	-	-	-	-	-	-	-	-

M-*Macrophomina* sp., A –*Alternaria* sp., C –*Colletotrichum* sp., N -*Nigrospora* sp., Asp.- *Aspergillus* sp., R.-*Rhizopus* sp., Cha.-*Chaetomium* sp., St -I Sterile aseptate and St -II –Sterile septate fungi culture

Table 2: Pathogenecity of isolated fungi on green gram

S. No	Name of the pathogen inoculated to seeds	Av. Undiseased Ungerminated seeds (%)	Av. pre-emergence rot (%)	Av. post-emergence rot (%)	Av. seed germination (%)	Decrease in germination over control (%)
1	*M.phaseolina.*	1.28 (0.00)	33.89 (31.11)	30.08 (25.11)	41.51 (43.77)	51.18
2	*A.alternata*	1.28 (0.00)	31.34 (27.66)	25.89 (19.22)	47.31 (53.11)	40.77
3	*C. capsici*	1.28 (0.00)	28.72 (23.33)	26.62 (20.11)	49.01 (56.56)	36.92
4	*N.sphaerica*	1.28 (0.00)	22.04 (14.11)	18.55 (10.11)	60.63 (75.77)	15.49
5	*Chaetomium* sp.	1.28 (0.00)	20.34 (12.22)	16.52 (8.11)	63.44 (80.13)	10.63
6	*Aspergillus* sp.	1.28 (0.00)	24.26 (16.77)	21.19 (13.11)	56.90 (70.11)	21.80
7	*R.oryzae*	1.28 (0.00)	21.19 (13.22)	18.50 (10.11)	61.34 (77.22)	13.87
8	Sterile aseptate fungi	1.28 (0.00)	18.53 (10.11)	16.48 (8.22)	64.90 (82.12)	8.41
9	Sterile septate fungi	1.28 (0.00)	18.52 (10.11)	17.51 (9.11)	64.16 (81.10)	9.55
10	Mixture of all the fungi	1.28 (0.00)	53.19 (64.22)	32.44 (28.57)	15.46 (7.21)	91.95
11	Healthy seeds without inoculation	19.56 (10.33)	1.28 (0.00)	1.28 (0.00)	71.24 (89.66)	-
	S.Em.±	0.05	0.50	0.56	1.00	
	C.D. at 5%	0.17	1.47	1.66	2.95	
	C.V. %	3.47	3.51	4.79	3.21	

Figures in parenthesis are original values; Figures outside the parenthesis are arcsine transformed values

Table 3: Symptoms observed during pathogencity test on seeds and plants of greengram

Symptoms	Fungi inoculated to seed										
	Mp	Aa.	Cc.	Ns.	Cha. sp.	Asp. sp.	Rhi. sp	St -I	St -II	M	H
	At 10 days										
Seed rot	+	+	+	+	+	+	+	+	+	+	-
Seedling necrosis	+	+	+	+	-	-	-			+	
Damping off of emerged seedling	+	+	+	+	+	+	+	+	+	+	-
Lesions of brown to red on cotyledonary leaves	+	+	+	-	-	-	-	-	-	+	-
Ungerminated undiseased seeds or healthy seedlings /plants	-	-	-	-	-	-	-	-	-	-	+
	At 25 days										
Leaf spot symptoms	+	+	+	-	-	-	-	-	-	+	-
	At 45 days										
Leaf blight	+	-	-	-	-	-	-		-	+	-
Root rot	-	-	-	-	-	-	-		-	+	-

Mp-*M. phaseolina*, Aa -*A. alternata*, Cc -*C. capsici*, Ns -*Nigrospora sphaerica*, Cha. sp. *Chaetomium* sp.,Asp. sp. *Aspergillus* sp., Rhi.sp.-*Rhizopus* sp., St -I -Sterile aseptate and St -II –Sterile septate fungi culture. M-mixture of all the fungi, H- healthy seed + Presence of the symptoms-Absence of the symptoms

Conclusion

Seed discolouration and seed abnormality was occurred in greengram seeds due to infection of single or group of seed infecting fungi. Seed infecting fungi were responsible for causing direct loss with decreasing seed germination and seed to seedling transmission of various seed borne diseases in greengram. Thus a suitable approach for the management of seed infecting fungi is strongly recommended.

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