CIBTech Journal of Microbiology ISSN: 2319-3867 (Online)
An Online International Journal Available at http://www.cibtech.org/cjm.htm
2013 Vol. 2 (2) April-June, pp.39-44/ Sharma et al.
Research Article

EFFECTS OF MICROFLORA ASSOCIATED WITH OKRA (*ABELMOSCHUS ESCULENTUS* L.) MOENCH SEEDS AND THEIR PHYTOPATHOLOGICAL EFFECTS

***Sharma D.K., Jain V.K., Jain R. and Sharma N.**
Botany Research lab, P.G. Department of Botany, Agrawal P.G. College, Jaipur-302 003, Rajasthan, India
**Author for Correspondence*

ABSTRACT
Eighty nine seed samples of okra (*Abelmoschus esculentus* L.) Moench were collected from 15 major okra growing districts of Rajasthan and subjected to dry seed examination. Various types of discoloured and deformed seeds including asymptomatic seeds *viz*. sclerotial seeds, dark brown seeds, white crusted seeds, spotted discoloured seeds, distorted or shriveled seeds and cracked or insect damaged seeds were observed. The seeds with water soaked symptoms, purple spots on seed surface and cotyledons were also observed and on incubation such seeds yielded bacteria.
On incubation, about 32 fungal and 5 bacterial species were observed. Out of them the dominant fungal and bacterial species are *Alternaria alternata, Arthrobotrys superba, Aspergillus flavus, A. niger, Cladosporium oxysporium, Curvularia lunata, Drechslera* spp., *Fusarium* spp., *Rhizoctonia bataticola* and *Rhizopus nigricans* and bacterial species as *Ralstonia solanacearum* (Smith) Yabuuchi *et al., Pseudomonas syringae* var. *syringae* van Hall and *Xanthomonas axonopodis* var. *malvacearum* (Smith) Vauterin. This microflora severely affecting seeds germination and cause many seedling abnormalities like failure or delayed seeds germination, bacterial oozing, stunting, rotting, wilting, and puffing of seedlings, collapse of hypocotyls and cotyledonary leaves that resulting seedling mortality.

INTRODUCTION
Okra belongs to family malvaceae an important vegetable crop. The plant is an erect, coarse, robust annual herb up to 2 m tall. Flowers are borne singly in the leaf axile on peduncles having a typical malvaceous floral organization with eight to ten very narrow, hairy, bracteoles forming an epicalyx. The fruits are long (10-30 cm), beaked, ridged, more or less oblong hairy capsules that dehiscing longitudinally.
The edible portion of the fruit, on average, contains approximately; 86.1% moisture, 9.7% carbohydrates, 2.2% protein, 1.0% fibers, 0.2% fats, and 0.9 % ash. The ripe seeds contain approximately 20% edible oil. Okra is a good source of vitamins A, B, C and minerals, especially Iodine. A mucilage preparation from the fruit can be used as a plasma replacement or blood volume expander (Kochhar, 2004).
Crop suffers from a number of phytopathogenic fungal and bacterial species causing severe losses, reduces plating and market value of okra seeds (Schaad and Kendrick, 1975, Neerguard, 1977, 1986**,** Bradbury, 1986, Schaad, 1989, Richardson, 1990, Agrawal, 2000).
Since the seeds are planting material therefore aim of present investigation is to study the major seed disorders due to microflora associated with seeds and their effect on them.

MATERIALS AND METHODS
Eighty nine seed samples of okra (*Abelmoschus esculentus* L.) Moench were collected from 15 different districts of Rajasthan in year 2011and 2012 to study the diversity and incidence of microflora and their effect. The seeds were subjected to dry seed examination, Standard Blotter Method (SBM) and agar plate method (Anonymous, 1996). For dry seed examination 400 seeds were drawn at random and examined by naked eye as well as by Nikon stereo binocular microscope and occurrence and percent incidence was evaluated. 100 untreated and 100 pretreated seeds with available 2% aqueous sodium hypochlorite were used for incubation test. Percentage of seed germination, seed-borne microflora, seedling symptoms and

other abnormalities were recorded on 8th day of incubation. Associated fungi and bacterial species were isolated, identified and percent loss was estimated. The bacterial species were identified by using semi-selective media.

RESULTS AND DISCUSSION

Dry Seed Examination

Eight nine seed samples when examined under stereo binocular microscope showed presence of dark brown or black discoloured seeds with mycelial crust 14 (1.50-6.75), sclerotial seeds (mycelial bodies) 24 (03.50-17.25%), white mycelial crusted seeds 17 (1.25-25.75%), spotted brown discoloured seeds 42 (1.25-18.25), distorted or shrivelled seeds with white crust 41 (0.25-6.25%) and Insect damaged or cracked seeds 50 (1.25-4.75%). The seeds with white crust, water soaked, shrivelled and discoloured symptoms 89 (07.50-37.75).

Fungi produce disease symptoms on seeds but detailed investigation as seed diseased as symptoms produced by pathogens (Neerguard, 1977, 1986). Gupta, Sindu and Naaz (1989) observed darker paleness on the surface of okra seeds at micropylar end caused by *Alternaria alternata, Drechslera* sp., *Cuvularia lunata* and *Aspergillus* spp.

Incubation Test

All eighty nine samples were tested using Standard Blotter Method (SBM) and agar medium test. A total of 32 fungal species of 20 genera were recorded. Among them the dominant fungi are *Alternaria alternata* (01-33%), *Arthrobotrys superba* (01-35%), *Aspergillus flavus* (01-36%), *Aspergillus niger* (01-13%) *Curvularia lunata* (02-24%), *Drechslera helodes* (01-18%), *Fusarium oxysporum* (01-35%), *Pencillium* sp. (01-20%), *Rhizoctonia bataticola* (01-30%) and *Rhizopus nigricans* (01-15%).

Chlorine pretreatment with 2% aqueous sodium hypochlorite for 2 min reduces the incidence of saprophytic fungus and enhances seed germination. Fungi that showed reduction in their incidence after chlorine pretreatment were *Alternaria alternata* (1-33 to 1-28%), *Arthrobotrys superba* (1-35 to 1-25%), *Aspergillus flavus* (1-36 to 1-25%), *Drechslera helodes* (01-18% to 01-14%), *Fusarium oxysporum* (1-35 to 01-29%), *Pencillium* sp. (1-20 to 1-12%), *Rhizoctonia bataticola* (1-30 to 1-27%) are *Rhizopus nigricans* (1-15 to 1-11%).

Phytopathological Effects

Most of the fungi associated with okra seeds caused adverse effect to seed germination and resulted in symptomatic seedlings. Seeds germination ranged from 1-83% in untreated and 10 to 100% in pretreated seeds in SBM. Some seeds remain ungerminated (due to oozing and rotting) or showed poor and delayed germination. Fungal species that hamped the seed germination were *Alternaria, Aspergillus, Chaetomium, Drechslera, Cladosporium, Curvularia, Fusarium, Penicillium, Rhizopus nigricans* and *Rhizoctonia bataticola*.

Fungi produced various symptoms and abnormalities on seedlings besides affecting seeds germination. Symptoms due to *Alternaria alternata* were brown black streaks on hypocotyls, browning of radicle and seedling mortality. *Aspergillus flavus* caused brown to black lesions on hypocotyls, browning of radicle and seedling mortality. Infection of *Fusarium oxysporum* and *F. equisetii* showed yellowing, drying and wilting. *Rhizoctonia bataticola* caused seed rot, blackening of radicle and hypocotyls. The tissue of hypocotyl and radicle became water soaked, seedling remain stunted that finally rotted.

Dwivedi and Tandon (1976) reported nine fungal species in bottle gourd viz. *Aspergillus flavus, A. niger, Alternaria alternata, Fusarium oxysporum* and *Rhizopus nigricans* associated with seed coat and cotyledons. *Alternaria alternata* causes shrivelling and discolourations in seeds, loss in seed germination and seedling blight in guar (Rangaswami and Rao, 1957), in cumin (Rastogi, 1993) and in chilli (Chitkara, Singh and Singh, 1986 a).

Shakir and Mirza (1992) reported that *Macrophomina phaseolina* was the most pathogenic in germination trials in cucurbits. Both sclerotia and pycnidia of *M. phaseolina* are found on seed surface that predominantly reduce the seed quality (Suryanaryan, 1963 and Pandey and Gupta, 1986). Similar

observations were reported in chilli (Bhale, Bhale, Pandey, and Pandey, 2000, Panwar and Vyas, 1974), moth bean and cowpea (Verma, 1990). *Rhizoctonia bataticola* causing dry rot, leaf blight and charcoal rot in cluster bean (Singh, 1953, Mihali and Alcorn, 1986), cucurbits (Maholay, 1989, Maholay and Sohi, 1985, Chandi and Maheshwari, 1992)) and in chilli (Chitkara, Singh and Singh, 1986 b).

Seeds with white crust, water soaked, shrivelled and discoloured symptoms were observed in okra and such seeds on incubation yielded bacteria. Same symptoms were also observed in chilli and tomato (Agrawal and Sharma, 2005, 2006, Sharma, 2007, Sharma and Agrawal, 2010), cluster bean (Jain and Agrawal, 2011), cowpea (Okechukwu, Ekpo, and Okechukwu, 2010)), chickpea (Reddy, Ahmed, and Verma,1986), crucifer seeds (Schaad, and Kendrick, 1975, Schaad, 1989), rape and mustard seeds (Sharma, Agrawal and Singh, 1992, 2002), pigeonpea (Sharma, Kumar, Agrawal, Singh, and Singh, 2001), cabbage (Bandyopadhyay, and Chattopadhyay, 1985, Siraree, Negi, and Kumar, 2008), sunflower (Ataga, and Akueshi, 1986, Godika, Agrawal, and Singh, 2000) and black gram (Agrawal, Jain, Sharma, and Jain, 2010) and okra (Agrawal, 2000, Sharma, Jain, Shafkat and Agrawal, 2012).

The 32 fungi isolated Actinomycetes, *Arthrobotrys supberba, Aspergillus fumigates, Cladosporium oxysporum, Drechslera* sp., *Fusarium moniliforme, Stachyobotrys* spp., *Verticillium alboatrum* and 3 bacteria namely *Ralstonia solanacearum* (Smith) Yabuuchi *et al., Pseudomonas syringae* var. *syringae* van Hall and *Xanthomonas axonopodis* var. *malvacearum* (Smith) Vauterin have not been reported earlier from okra seeds.

Table 1: Incidence of various disorders in dry seed examination and microflora association with them in standard blotter method in seeds of okra

Type of seed discolouration	Occurrence	Incidence (RPO)	Important microorganism associated with seeds
Brown or black discoloured seeds with mycelial crust	14	01.50-06.75	*Curvularia* spp. *Drechslera* spp. and *Alternaria* spp.
Sclerotial seeds (mycelial bodies)	24	03.50-17.25	*R. bataticola, Aspergillus* spp
White mycelial crusted seeds	17	01.25-25.75	*Fusarium oxysporum, F. moniliforme,* Actinomycetes spp.
Spotted brown discoloured seeds	42	01.25-18.25	*Alternaria* sp. *Curvularia* sp. *Chaetomium* and *Myrothecium* spp.
Distorted or shrivelled seeds with white crust	41	0.25-06.25	*Aspergillus* spp., *Penicillium* spp., *Fusarium* sp.
Insect damaged or cracked seeds	50	01.25-04.75	*Aspergillus* spp., *Chaetomium* spp., *Pencillium* spp. *Rhizopus* spp., Insects and eggs.
Seeds with White crust, water soaked, shrivelled and discoloured	89	07.50-37.75	*Ralstonia solanacearum , Pseudomonas syringae* var. *syringae* and *Xanthomonas axonopodis* var. *malvacearum*

Table 2: Percent range of important microflora in untreated and pretreated seeds in standard blotter method and their associated seedling symptom

Fungi	Untreated			Pretreated			Phytopathological effects
	Occurrence	RPO	% Range	Occurrence	RPO	% Range	
Actinomycetes	13	**14.60**	01-10	05	**05.61**	01-6	delayed germination
Alternaria alternata	30	**33.70**	01-33	24	**26.96**	1-28	Leaf blight and leaf spot
Arthrobotrys superba	22	**24.71**	01-35	13	**14.60**	1-25	Seed rotting
Aspergillus flavus	33	**37.07**	01-36	17	**19.10**	1-25	Yellowing and rotting of seedling
Aspergillus niger	30	**33.70**	01-13	04	**04.49**	1-09	
Chaetomium globosum	16	**17.97**	01-25	13	**14.60**	1-19	Seed rotting
C. spinosum	13	**14.60**	01-17	09	**10.11**	1-12	Seed rotting
Curvularia lunata	19	**21.34**	02-24	16	**17.97**	1-20	Leaf spot and rotting
Drechslera helodes	10	**11.23**	01-18	08	**08.98**	1-14	Leaf spot and rotting
Fusarium moniliforme	16	**17.97**	01-19	12	**13.48**	1-08	Die back, wilting and damping
F. oxysporum	30	**33.70**	01-35	26	**29.21**	1-29	
Penicillium sp.	20	**22.47**	01-20	15	**16.85**	1-12	
Rhizoctonia bataticola	23	**25.84**	01-30	22	**24.71**	1-27	Seed rot blackening of radicle and hypocotyls, stunted seedling
Rhizopus nigricans	15	**16.85**	01-15	11	**12.35**	1-11	Collar rot , root rot and seed rot
Verticillium alboatrum	07	**07.86**	01-50	05	**05.61**	1-9	Wilting, root rotting and browning; leaf spot and leaf blight, seedling rottings
Ralstonia solanacearum	87	**97.75**	15-92	83	**93.25**	5-80	
Pseudomonas syringae var. *syringae*	62	**69.66**	12-72	59	**66.29**	6-68	
Xanthomonas axonopodis var. *malvacearum*	41	**46.06**	10-69	35	**39.32**	5-65	

ACKNOWLEDGMENT
One of the authors (Dr. D.K. Sharma) is thankful to UGC-CRO, Bhopal for providing the financial assistance to carry out the work.

REFERENCES
Agrawal K and Sharma DK (2007). Seed borne infection of *Pseudomonas syringae* pv. *syringae* in chilli, its phytopathological effects and disease management in International Seed Testing Association (ISTA). In ISTA Seed Symposium,Brazil.
Agrawal K and Sharma DK (2005). Seed-borne infection of *Xanthomonas campestris* pv. *vesicatoria* in chilli, its phytopathological effects and disease management. In: Indian Society of Mycology and Plant Pathology. Global Conference II November 25-29Udaipur, Rajasthan.
Agrawal K, JainV K, Sharma DK and Jain R (2010). Ecofriendly control of seed-borne fungal diseases in black gram. Edt Singh, T and Agrawal, K Aaviskar publishers, distibutors, Jaipur 126-130.
Agrawal S (2000). Seed-borne and post-harvest disease of okra (*Abelmoschus esculentus* Monech.). PhD Thesis, University of Rajasthan, Jaipur, 181.
Annonymous (1996). International rules of seed testing. International Seed Testing Association. *Seed Science and Technology* 13 299-513.
Ataga AE and Akueshi C O (1986). Changes in oil and free fatty acid contents of sunflower seeds inoculated with *Alternaria tenuis* Auct., *Curvularia lunata* (Wakker) Boedijn, *Fusarium moniliforme* Sheld. And *Macrophomina phaseolina* (Tassi) Goid. *Phytopathologia Mediterranal* **25** (1-3) 44-46.
Bandyopadhyay S and Chattopadhyay S B (1985). Incidence of black rot of cabbage and cauliflower under different conditions of infection. *Indian Journal of Agricultural Science* **55** 350-354.
Bhale U, Bhale M S, Pandey B R and Pandey RP (2000). Seed-borne fungi of chilli in M.P. and their significance. *Journal of Mycopathological Research* **38**(2) 117-119.
Bradbury JF (1986). Guide to plant pathogenic bacteria. CAB International Mycological Institute (CMI), UK, 332.
Chandi R and Maheshwari SK (1992). Seed mycoflora of bottle gourd and their control. *Agriculture Science Digest* **12**(2) 79-81.
Chitkara S, Singh T and Singh D (1986a). *Alternaria tenuis* in chilli seeds of Rajasthan. *Biological Bulletin of Indian* **8** 18-23.
Chitkara S, Singh T and Singh D (1986b). *Rhizoctonia solani* in chilli seeds of Rajasthan. *Indian Phytopathology* **39** 290-291.
Dwivedi RS and Tandon RN (1976). Studies on mycoflora of stored seeds of bottle gourd. *Acta Botanica Indica* **4**(2) 139-143.
Godika S, Agrawal K and Singh T (2000). Histopathological and biochemical changes in seeds of sunflower infected with *Pseudomonas syringae.* In: *Indian Phytopathology*. Golden Jubilee-Proceedings: 1131-1132.
Gupta KK, Sindu IR and Naaz S (1989). Seed mycoflora of *Abelmoschus esculentus* (*L*.) Moench survey enumeration. *Acta Botanica Indica* **17**(2) 200-206.
Jain R and Agrawal K (2011). Incidence and seed transmission of *Xanthomonas axonopodis*pv. *cyamopsidis* in cluster bean. *Journal of Agricultural Technology* **7**(1) 197-205.
Kochhar SL (2004). Economic Botany in the tropics. Macmillan India Limited, Daryaganj, New Delhi, 604.
Maholay MN (1989). Seed-borne diseases of cucurbits. Seeds and Farms, **15**(2) 30-31.
Maholay MN and Sohi HS (1985). *Macrophomina* seed rot of bottle gourd, squash and muskmelon. *Indian Journal of Mycology and Plant Pathology* **13**(2) 192-197.
Mihail JD and Alcorn SM (1986). *Macrophomina phaseolina* from guar in Arizona. *Canadian Journal of Botany* **64** 11-12.
Neergaard P (1977). *Seed Pathology*. The Macmillan Press Ltd., London, 1187.

Neergaard P (1986). *Seed Pathology* (Abridged edt.). Published by S. Chand & Company Ltd., New Delhi, 466.
Okechukwu R U, Ekpo E J A and Okechukwu O C (2010). Seed to plant transmission of *Xanthomonas campestris* pv. *vignicola* isolates in cowpea, *African Journal of Agricultural Research* **5** (6) 431- 435.
Pandey KN and Gupta RC (1986). Inhibitory effect of fungal metabolite on germination and sprouting of cucurbit seeds. *Indian Journal of Mycology and Plant Pathology* **16**(3) 253-256.
Panwar KS and Vyas NL (1974). *Cladosporium oxysporum* causing fruit rots of *Punica granatum, Zizyphus jujuba* and *Capsicum annuum. Indian Phytopathology* **27** 121-22.
Rangaswami G and Rao AV (1957). Alternaria blight of cluster beans. *Indian Phytopathology* **10**(1) 18-25.
Rastogi A (1993). Occurance and transmission of *Alternaria burnsii* in cumin seeds grown in Rajasthan. *Journal of the Indian Botanical Society* Abstracts **72** 151-154.
Reddy J R, Ahmed K M and Verma B K (1986). Bacterial wilt of chickpea caused by *Xanthomonas campestris* (Pam.) Dowson, International Chickpea Newsletters, **19** 13-15.
Richardson M J (1990). An annotated list of seed-borne diseases **4**. *Proceedings of the International Seed Testing Association* Zurich, Switzerland.
Schaad N W (1989). Detection of *Xanthomonas campestris* pv. *campestris* in crucifer, In: detection of bacteria in seed and another planting material 68 – 75.
Schaad N W and Kendrick R (1975). A qualitative method for detecting *Xanthomonas campestris* in crucifer seed, *Phytopathology* **65** 1034 – 1036.
Shakir AS and Mirza JH (1992). Seed-borne fungi of bottle gourd from Faisalabad and their control. *Pakistan Journal of Phytopathology* **4**(1-2) 54-57.
Sharma D K (2007). Seed-borne and post-harvest bacterial diseases of chilli (*Capsicum* spp.) and tomato (*Lycopersicon esculentum* Mill.) crops and their management. PhD Thesis, University of Rajasthan, Jaipur, 237.
Sharma DK and Agrawal K (2010). Incidence and Histopathology of *Ralstonia solanacearum* in Tomato Seeds. *Journal of Mycology and Plant Pathology* **40**(1) 115-119.
Sharma DK, Jain VK, Shafkat R and Agrawal K (2012). Effect and seed-borne infection of *Pseudomonas syringae* var. *syringae* in okra (*Abelmoschus esculentus* L.) Moench and its non-hazardous management. National symposium on environment management and biodiversity conservation. Govt Lohiya P.G. College, Churu, 101.
Sharma J, Agrawal K and Singh D (1992). Detection of *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson infection in rape and mustard seeds, *Seed Research* **20** 128-133.
Sharma M, Agrawal K and Singh T (2002). Incidence and seed transmission of *Xanthomonas campestris* pv. *cajani, Journal of Mycology and Plant Pathology* **32** (1) 1-5.
Sharma M, Kumar D, Agrawal K, Singh T and Singh D (2001). Colonization of pigeonpea seed by *Xanthomonas campestris* pv. *cajani, Journal of Mycology and Plant Pathology* **31** (2) 216-219.
Singh RS (1953). A leaf blight of guar. *Current Science* **19** 155-156.
Siraree A, Negi Y K and Kumar J (2008). Detection of seed-borne inoculum of *Xanthomonas campestris* pv. *campestris,* the black rot pathogen of cabbage and estimation of genetic diversity, *Journal of Mycology and Plant Pathology* **38** (1) 65-72.
Suryanarayan (1963). Sclerotia on seeds of bottle gourd. *Indian Journal of Horticulture* **20** 141.
Verma R (1990). Studies on seed-borne mycoflora and disease of moth bean and cowpea grown in Rajasthan. PhD Thesis, University of Rajasthan Jaipur.